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L4 ANSWER 1 OF 21 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2004:80972 The Genuine Article (R) Number: 762WM. TCR vaccines against a murine T cell lymphoma: A primary role for **antibodies** of the IgG2c class in tumor protection. Lambert S L; Okada C Y; Levy R (Reprint) . Stanford Univ, Sch Med, Dept Med, Div Oncol, CSR 1126, 269 Campus Dr, Stanford, CA 94305 USA (Reprint); Stanford Univ, Sch Med, Dept Med, Div Oncol, Stanford, CA 94305 USA; Stanford Univ, Sch Med, Program Immunol, Stanford, CA 94305 USA; Vet Affairs Med Ctr, Res Serv, Div Hematol & Oncol, Ann Arbor, MI 48105 USA; Univ Michigan, Ann Arbor, MI 48105 USA. JOURNAL OF IMMUNOLOGY (15 JAN 2004) Vol. 172, No. 2, pp. 929-936. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. ISSN: 0022-1767. Pub. country: USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Tumor-associated proteins can act as effective immunotherapeutic targets. Immunization with tumor TCR protein conjugated to the immunogenic protein keyhole limpet hemocyanin (**KLH**) protects mice from tumor challenge with the marine T cell lymphoma C6VL. The immune mechanisms responsible for this tumor protection are of interest for designing more effective vaccine strategies. Previous studies using depletion experiments had suggested a CD8-mediated component of protection induced by TCR-**KLH** vaccines. In this study we used CD8alpha knockout, muMT, and FcgammaR knockout mice to investigate the relative roles of CD8(+) T cells and Ab in protective immunity induced by TCR-**KLH** immunization. We found that CD8(+) T cells are not required for tumor protection, although they may contribute to protection. Vaccine-induced Abs are sufficient to mediate protection against this murine T cell lymphoma through an FcR-dependent mechanism. This was confirmed with Ab transfers, which protect challenged mice. Additionally, recombinase-activating gene 1(-/-) splenocytes can mediate Ab-dependent cellular cytotoxicity against this tumor in the presence of bound anti-TCR Absolute IFN-gamma knockout mice demonstrated a requirement for IFN-gamma, probably via generation of IgG2c

Abs, in vaccine-induced tumor protection. IFN-gamma knockout mice were not protected by immunization and had a severe impairment in IgG2c Ab production in response to immunization. Although mock-depleted anti-TCR Abs could transfer tumor protection, IgG2c-deficient anti-TCR Abs were unable to transfer tumor protection to wild-type mice. These results suggest that TCR-**KLH** vaccine-induced tumor protection in the C6VL system is primarily attributable to the induction of IgG2c Abs and Immoral immunity. The Journal of Immunology, 2004, 172: 929-936.

L4 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
2003:319446 Document No. 138:336413 Covalently reactive transition state analogs (CRTSA) of **antibody** for treating autoimmune, microbial, lymphoproliferative and neoplastic diseases and for screening phage display library and B cell-expressing surface **antibody**. Paul, Sudhir; Nishiyama, Yasuhiro (USA). U.S. Pat. Appl. Publ. US 2003078203 A1 20030424, 52 pp., Cont.-in-part of U.S. Ser. No. 862,849. (English). CODEN: USXXCO. APPLICATION: US 2002-114716 20020401. PRIORITY: US 1998-46373 19980323; US 2001-PV280624 20010331; US 2001-862849 20010522.
AB The CRTSA of **antibodies** or catalytic **antibodies** comprise an epitope of a target protein antigen, an electrophilic covalently reactive center bearing a partial or full neg. charge and an electron withdrawing (or donating) substituent optionally containing a flanking peptide sequence. The provided CRTSA are useful for production, selection and inhibition of catalytic **antibodies** specific to tumor necrosis factor, epidermal growth factor receptor, interleukin 1, gp120, gp160, gag, pol, HBsAg, bacterial exotoxin, EGF, TGFα, p53, prostate-specific antigen, CEA, prolactin, hCG, c-myc, c-fos, c-jun, HER-2, prolactin receptor, steroid receptor and interleukin 4. The CRTSA **antibodies** are therefore useful as vaccine or for passive immunotherapy of autoimmune diseases, lymphoproliferative diseases, microbial infection,. The CRTSA **antibodies** are also useful for screening phage displaying or B cell expressing catalytic **antibodies** on the surface.

L4 ANSWER 3 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
2002:142551 Document No. 136:198916 Chimeric **antibodies** for anti-idiotype therapy of B cell disorders. Gold, Daniel P.; Shopes, Robert J. (Favrille, Inc., USA). PCT Int. Appl. WO 2002013862 A2 20020221, 100 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US25204 20010810. PRIORITY: US 2000-PV224723 20000811; US 2000-PV224722 20000811; US 2001-PV279079 20010323.
AB The authors disclose a method for treatment of B-cell tumors and autoimmune disease. This method comprises administering chimeric proteins. For example, a chimeric protein may be composed of either the VH or VL region of a tumor-associated Ig fused to a heterologous Ig constant region. The genes encoding VH and/or VL regions and the genes encoding Ig constant regions are isolated and inserted into an expression vector. The chimeric proteins are produced by introducing the expression vectors into insect cell lines. The chimeric proteins are purified using affinity chromatog. and then conjugated to an immunogenic carrier, keyhole-limpet hemocyanin (**KLH**). In one example, immunization with chimeric **antibody** induced regression of tumor masses in human non-Hodgkin's lymphoma.

L4 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
2002:142550 Document No. 136:198935 Chimeric protein comprising TCR

Vα/Vβ and immunoglobulin constant region for immunotherapy of T cell-mediated diseases. Gold, Daniel P.; Shopes, Robert J. (Favrille, Inc., USA). PCT Int. Appl. WO 2002013861 A2 20020221, 110 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US25203 20010810. PRIORITY: US 2000-PV224723 20000811; US 2000-PV224722 20000811; US 2001-PV266133 20010201.

AB The present invention provides a method for altering a T cell mediated pathol. in a patient. This method comprises administering a composition comprising at least one and/or two chimeric proteins. Each chimeric protein comprises at least a portion of either the Vα or Vβ region of a TCR from particular T cells from a patient having a T cell mediated pathol., and an Ig constant region. The genes encoding Vα and/or Vβ regions and the genes encoding Ig constant regions are isolated and inserted into an expression vector. The chimeric proteins are produced by introducing the expression vectors into insect cell lines. The chimeric proteins are purified using **antibody** affinity columns, and then chemical conjugated to an immunogenic carrier, keyhole-limpet hemocyanin (**KLH**). Since the conjugates comprises chimeric proteins made specifically from particular T cells from a patient having T cell mediated pathol., when it is administered to such a patient, with or without a cytokine, such as granulocyte-macrophage-CSF, or a chemokine, it can induce immune responses to alter such a T cell mediated pathol.

L4 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN

2002:272796 Document No. 136:293510 Anti-allergic vaccines comprising peptides of Fc portion of IgE ε heavy chain and carrier protein. Morsey, Mohamad Ali; Sheppard, Michael George; Wheeler, David Walter (Pfizer Products Inc., USA). Eur. Pat. Appl. EP 1195161 A2 20020410, 38 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2001-307247 20010824. PRIORITY: US 2000-PV228989 20000830.

AB The present invention provides compns. and methods for the use of antigenic peptides derived from the Fc portion of the epsilon heavy chain of an IgE mol. as vaccines for the treatment and prevention of IgE-mediated allergic disorders. In particular, the invention provides compns., methods for the treatment and prevention of IgE-mediated allergic disorders comprising an immunogenic amount of one or more antigenic peptides derived from the CH3 domain or junction of Ch-3/CH4 domain of an IgE mol. and methods for the evaluation of IgE mediated allergies in dogs. The allergic disorder is asthma, allergic rhinitis, gastrointestinal allergy, food allergy, eosinophilia, conjunctivitis, or glomerular nephritis. The vaccine compns. may also comprises carrier protein such as **KLH**, PhoE, rmLT, TraT and gD from BhV-1 virus; and adjuvant such as aluminum hydroxide, monophosphoryl lipid A, Thr-MDP, immunostimulatory oligonucleotide, cytokine, interleukin 12, interleukin 2, interleukin 1, saponin, cholera toxin, heat labile toxin, etc.

L4 ANSWER 6 OF 21 MEDLINE on STN

2002408469. PubMed ID: 12162820. lacZ transgenic rats tolerant for beta-galactosidase: recipients for gene transfer studies using lacZ as a reporter gene. Menoret Severine; Aubert Dominique; Tesson Laurent; Braudeau Cecile; Pichard Virginie; Ferry Nicolas; Anegon Ignacio. (INSERM U437 and ITERT, 44093, Nantes, France.) Human gene therapy, (2002 Jul 20) 13 (11) 1383-90. Journal code: 9008950. ISSN: 1043-0342. Pub. country:

United States. Language: English.

AB Gene transfer of reporter genes may trigger immune responses against the heterologous protein resulting in shortening of gene expression and inflammation. We generated transgenic rats expressing the lacZ gene under the control of the human immunodeficiency virus type 1 (HIV-1) long-terminal repeat (LTR) (HIV-lacZ) to obtain rats with undetectable transgene expression using histologic methods, thus avoiding interference with beta-galactosidase (beta-gal) expression from gene transfer, and displaying immune tolerance toward beta-gal. LacZ transgenic mice with tolerance toward beta-gal have already been used for gene transfer but rats constitute unique animal models with several advantages compared to mice. Two transgenic lines displayed low levels of beta-gal mRNA in most organs tested, as detected only by reverse transcription-polymerase chain reaction (RT-PCR). The protein was undetectable by immunohistology and was only detected in the thymus and spleen using a sensitive enzyme-linked immunosorbent assay (ELISA). HIV-lacZ transgenic rats displayed immune tolerance to beta-gal because immunization with beta-gal resulted in markedly lower cellular and **antibody** responses compared to wild-type controls, whereas immunization with a nonrelated antigen, keyhole limpet hemocyanin (**KLH**), resulted in comparable immune responses. The usefulness of this model in gene transfer was tested using a retroviral vector, which does not elicit destructive immune responses against transduced cells. Retroviral-mediated nlslacZ gene transfer in the liver resulted in nuclear beta-gal expression for longer than 12 months in HIV-lacZ transgenic rats, whereas wild-type controls showed nuclear beta-gal expression for less than 1 month. After gene transfer of nlslacZ to the liver, **antibodies**, cytotoxic T lymphocytes (CTLs), and proliferation against beta-gal were detected in wild-type controls but not in HIV-lacZ transgenic rats. In conclusion, HIV-lacZ transgenic rats displaying low beta-gal expression and immune tolerance toward beta-gal are a useful tool to analyze the spatial and temporal expression of the beta-gal protein in gene transfer experiments using lacZ as a reporter gene.

L4 ANSWER 7 OF 21 MEDLINE on STN DUPLICATE 1

2002310254. PubMed ID: 12052563. Enhancement of the protective efficacy of an oprF DNA vaccine against Pseudomonas aeruginosa. Price Brian M; Barten Legutki J; Galloway Darrell R; von Specht Bernd Ulrich; Gilleland Linda B; Gilleland Harry E Jr; Staczek John. (Department of Microbiology, The Ohio State University, 484 West 12th Ave., Columbus, OH 43210-1292, USA.) FEMS immunology and medical microbiology, (2002 Jun 3) 33 (2) 89-99. Journal code: 9315554. ISSN: 0928-8244. Pub. country: Netherlands. Language: English.

AB The outer membrane protein F gene (oprF) of Pseudomonas aeruginosa was recently shown by us to protect mice from P. aeruginosa chronic pulmonary infection when used as a DNA vaccine administered by three biolistic (gene gun) intradermal inoculations given at 2-week intervals. In the present study, we used two different strategies to improve the protective efficacy of the DNA vaccine. In the first strategy, mice were primed with two biolistic intradermal inoculations with the oprF vaccine and then were given a final intramuscular booster immunization containing either a synthetic peptide-keyhole limpet hemocyanin (**KLH**) conjugate or a chimeric influenza virus. Both the synthetic peptide conjugate and the chimeric virus contained peptide 10, a previously identified immunoprotective epitope of protein F. The second strategy involved the addition of a second outer membrane protein to the vaccine. DNA encoding a **fusion protein** comprised of the C-terminal half of protein F fused to OprI was administered by three biolistic intradermal inoculations. Challenge with P. aeruginosa in a chronic pulmonary infection model demonstrated that boosting with the chimeric virus (but not with peptide-**KLH**) or adding oprI to the DNA vaccine significantly enhanced protection as compared to that afforded by the oprF vaccine given alone. Thus, both strategies appear to augment the

protection afforded by an oprF-only DNA vaccine.

L4 ANSWER 8 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
2001:565269 Document No. 135:148230 Sequences of MHC class II restricted T cell epitopes from the cancer antigen NY-ESO-1 and uses thereof in cancer immunotherapy. Wang, Rong-Fu; Rosenberg, Steven A.; Zeng, Gang (Government of the United States of America, as Represented by the Secretary, Department of Health and Human Services, USA). PCT Int. Appl. WO 2001055393 A2 20010802, 134 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US2765 20010126. PRIORITY: US 2000-PV179004 20000128; US 2000-PV237107 20000929.

AB The invention provides novel sequences of MHC class II restricted T cell epitopes derived from the cancer antigen NY-ESO-1 and provides strategies in cancer diagnosis, prevention and treatment. The novel MHC class II epitopes from NY-ESO-1 are recognized by CD4+ T lymphocytes in an HLA class II restricted manner, in particular HLA-DR or HLA-DP restricted. The epitopes are useful as a cancer vaccine to elicit an immunogenic response against cancers. The products of the gene are promising candidates for immunotherapeutic strategies for the prevention, treatment and diagnosis of patients with cancer,in particular, melanoma.

L4 ANSWER 9 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
2001:409263 Document No. 136:198459 Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus. Langeveld, J. P. M.; Brennan, F. R.; Martinez-Torrecuadrada, J. L.; Jones, T. D.; Boshuizen, R. S.; Vela, C.; Casal, J. I.; Kamstrup, S.; Dalsgaard, K.; Meloen, R. H.; Bendig, M. M.; Hamilton, W. D. O. (Institute for Animal Science and Health (ID-Lelystad), Lelystad, 8200 AB, Neth.). Vaccine, 19(27), 3661-3670 (English) 2001. CODEN: VACCDE. ISSN: 0264-410X. Publisher: Elsevier Science Ltd..

AB A vaccine based upon a recombinant plant virus (CPMV-PARVO1), displaying a peptide derived from the VP2 capsid protein of canine parvovirus (CPV), has previously been described. To date, studies with the vaccine have utilized viable plant chimeric particles (CVPs). In this study, CPMV-PARVO1 was inactivated by UV treatment to remove the possibility of replication of the recombinant plant virus in a plant host after manufacture of the vaccine. We show that the inactivated CVP is able to protect dogs from a lethal challenge with CPV following parenteral immunization with the vaccine. Dogs immunized with the inactivated CPMV-PARVO1 in adjuvant displayed no clin. signs of disease and shedding of CPV in feces was limited following CPV challenge. All immunized dogs elicited high titers of peptide-specific **antibody**, which neutralized CPV in vitro. Levels of protection, virus shedding and VP2-specific **antibody** were comparable to those seen in dogs immunized with the same VP2- peptide coupled to keyhole limpet hemocyanin (**KLH**). Since plant virus-derived vaccines have the potential for cost-effective manufacture and are not known to replicate in mammalian cells, they represent a viable alternative to current replicating vaccine vectors for development of both human and veterinary vaccines.

L4 ANSWER 10 OF 21 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:369845 The Genuine Article (R) Number: 312LY. Vaccination of high-risk breast cancer patients with mucin-1 (MUC1) keyhole limpet hemocyanin conjugate plus QS-21. Gilewski T (Reprint); Adluri S; Ragupathi G; Zhang S L; Yao T J; Panageas K; Moynahan M; Houghton A; Norton L; Livingston P O . MEM SLOAN KETTERING CANC CTR, DEPT MED, DIV SOLID TUMOR, BREAST CANC

SERV, 1275 YORK AVE, NEW YORK, NY 10021 (Reprint); MEM SLOAN KETTERING CANC CTR, DEPT MED, SERV IMMUNOL, DIV SOLID TUMOR, NEW YORK, NY 10021; MEM SLOAN KETTERING CANC CTR, DEPT EPIDEMIOL & BIOSTAT, NEW YORK, NY 10021. CLINICAL CANCER RESEARCH (MAY 2000) Vol. 6, No. 5, pp. 1693-1701. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202. ISSN: 1078-0432. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Our objective was to determine whether an immune response can be generated against MUC1 peptide and against tumor cell MUC1 after vaccination with MUC1-keyhole limpet hemocyanin (**KLH**) conjugate plus QS-21 in breast cancer patients.

Nine patients with a history of breast cancer but without evidence of disease were treated with MUC1-**KLH** conjugate plus QS-21, containing 100 mu g of MUC1 and 100 mu g of QS-21, s.c. vaccinations were administered at weeks 1, 2, 3, 7, and 19, Peripheral blood was drawn at frequent intervals to assess **antibody** titers, Skin tests were placed at weeks 1, 3, 9, and 21 to determine delayed type hypersensitivity reactions.

Common toxicities included a local skin reaction at the site of the vaccine, usually of 4-5 days' duration, and mild flu-like symptoms usually of 1-2 days' duration. High IgM and IgG **antibody** titers against synthetic MUC1 were detected. IgG **antibody** titers remain elevated from a minimum of 106-137 weeks after the first vaccination. Binding of IgM **antibody** to MCF-7 tumor cells was observed in seven patients, although there was minimal binding of IgG **antibody** . Two patients developed significant **antibody** titers post-highdose chemotherapy and stem cell reinfusion. There was no evidence of T cell activation.

This MUC1-**KLH** conjugate plus QS-21 was immunogenic and well tolerated in breast cancer patients. Additional trials are ongoing to determine the optimal MUC1 peptide for use in larger clinical trials. Further investigation of vaccine therapy in high-risk breast cancer is warranted.

L4 ANSWER 11 OF 21 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:33140 The Genuine Article (R) Number: 387ZH. Identification and characterization of a novel gene KE04 differentially expressed by activated human dendritic cells. Li N; Huang X; Zhao Z L; Chen G Y; Zhang W P; Cao X T (Reprint). Second Mil Med Univ, Dept Immunol, 800 Xiangyin Rd, Shanghai 200433, Peoples R China (Reprint); Second Mil Med Univ, Dept Immunol, Shanghai 200433, Peoples R China. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (20 DEC 2000) Vol. 279, No. 2, pp. 487-493. Publisher: ACADEMIC PRESS INC. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0006-291X. Pub. country: Peoples R China. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To better understand the molecular mechanisms of dendritic cells (DC) function, we isolated differentially expressed genes in Ag-activated DC by a PCR-based subtractive hybridization technique. A novel fall-length cDNA, KE04, was thus isolated from **KLH**-activated human PBMC-derived DC. KE04 cDNA encoded a 346-aa protein devoid of functionally indicative motifs, KE04 protein showed 64% identity with a Caenorhabditis elegans protein and 83% identity with a human putative protein. Distant relationship was also found with other prokaryotic and eukaryotic proteins. Differential expression of KE04 in activated DC other than nonactivated DC was confirmed at both mRNA and protein levels. KE04 mRNA expression was detectable in various human tissues and cell lines by Northern blot and RT-PCR, Western blot and confocal microscopy analysis indicated that its cytolocalization was intracellular. Our data suggest the potential involvement of KE04 in DC activation and will facilitate the research of molecular mechanism of DC function. (C) 2000 Academic Press.

L4 ANSWER 12 OF 21 MEDLINE on STN DUPLICATE 2

2000164756. PubMed ID: 10699579. Facile generation and use of immunogenic polypeptide fusions to a sparingly soluble non-antigenic protein carrier. Knuth M W; Okragly A J; Lesley S A; Haak-Frendscho M. (Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA.. mknuth@promega.com) . Journal of immunological methods, (2000 Mar 6) 236 (1-2) 53-69. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Many researchers attempt to prepare antipeptide **antibodies** by immunizing animals with preparations of **fusion proteins** or conjugates between the target peptide and a larger protein (such as GST or **KLH**). Often, the immune response to the larger protein dominates. We have engineered a protein to be sparingly soluble in aqueous solution and nonantigenic, and show that fusions of this sparingly soluble non-antigenic protein (SSNAP) to target peptide sequences can be purified easily to a point suitable for immunizations. When animals are immunized with such **fusion proteins**, the majority of the immune response is to the target peptide. In all three cases tested, the peptide-specific immune response generated using the SSNAP carrier was greater than that obtained with peptides chemically linked to BSA or **KLH**, or expressed as **fusion proteins** to GST.
The SSNAP carrier induced a very early IgG response with all classes of IgG well represented in the specific **antibody** response. All of the SSNAP fusion peptide-derived **antibodies** were capable of recognizing the full-length target protein in both ELISA and Western analysis. Based on the superior performance of the SSNAP antigens, these studies suggest this novel strategy will have broad utility for the generation of peptide **antibodies**.

L4 ANSWER 13 OF 21 EMBASE on STN

1999434380 EMBASE Biochemical basis of antigen-specific suppressor T cell factors: Controversies and possible answers. Ishizaka K.; Ishii Y.; Nakano T.; Sugie K.. K. Ishizaka, La Jolla Inst. for Allergy/Immunol., San Diego, CA, United States. Advances in Immunology 74/- (1-60) 2000.
Refs: 196.
ISSN: 0065-2776. CODEN: ADIMAV. Pub. Country: United States. Language: English. Summary Language: English.

AB It is now obvious that Ts cells express TCR and release suppressor factors which have identical epitope specificity and a common antigenic determinant with TCR. The antigen-specific T cell factors released from the Ts cells are most likely to be a derivative of TCR or a conjugate of TCR with some cytokine such as GIF. Binding of such TsF to nominal antigen can be explained by the fact that the T cell epitope for TCR on the cell source of TsF represents an external structure of the antigen. Considering that the TsF is a derivative of TCR, we suspect that TsF is specific for a processed antigenic peptide associated with a MHC product, but the peptide itself has some affinity for TsF and TCR. Major problems in the process of biochemical identification of TsF were that the concentration of TsF in culture supernatants of Ts cells is extremely low, and the factors are hydrophobic. It should be noted that either serum-free or protein-free culture medium was employed in all of the experiments in which TCR-like molecules in culture supernatants were biochemically identified. Another problem was that culture supernatants of Ts hybridomas contained several species of proteins which nonspecifically bound to Sepharose and/or AffiGel, and these proteins were recovered in acid eluates of immunosorbents. In all of our experiments, therefore, culture supernatants were preabsorbed by recycling the concentrated supernatants through a large column of rabbit-IgG(RGG)-coupled AffiGel overnight prior to affinity purification. The RGG-AffiGel removed several cell-derived proteins including inactive 13-kDa GIF peptide, and this procedure was essential for identification of the 55-kDa GIF, which bound both anti-GIF **antibodies** and anti-TCRα chain in immunoblotting.
Accumulated evidence for the relationship between TsF and TCR suggested

that TsF may be formed either by an alternative splicing of the TCR genes or by posttranslational modifications of TCR chain(s). In the case of the 55-kDa GIF, it is clear that alternative splicing of TCRα gene is not involved. The 55-kDa protein formed by a stable transfectant of a mutated cDNA encoding TCRα chain with histidine tag at C terminus bound to Ni-NTA agarose, indicating that the 55-kDa GIF contained the C terminus of TCRα chain. We believe that the 55-kDa GIF is a posttranslationally formed conjugate of TCRα chain with GIF. Evidence was presented that upon antigenic stimulation of Ts cells, GIF is translocated through ER and forms a conjugate with TCRα chain. The conjugate formation would protect TCRα chain from ER degradation, probably prevent association of CD3 complex with TCRαβ, and facilitate the secretion of the TCR-GIF conjugate through constitutive secretory pathway. Translocation of the GIF peptide through ER, followed by posttranslational modification of the peptide in ER/Golgi would transform the inactive GIF to bioactive GIF. Thus the Ts cells release antigen-specific GIF having bioactivity upon antigenic stimulation. The conjugate formation explains the reason why mRNA of the 55-kDa GIF does not exist even when the 55-kDa GIF is being formed by Ts cells. Lack of the mRNA of the 55-kDa GIF in Ts cells with explain why cDNA clone encoding TsF could not be obtained in spite of great efforts by several investigators. We believe that GIF plays important roles not only in the formation of a soluble form derivative of TCR but also in the function of TsF. We suspect that the TCR-GIF conjugate would bind to antigen- primed T cells and B cells through the high affinity receptors for bioactive GIF and exert immunosuppressive effects. The abilities of the 13-kDa bioactive GIF to suppress antigen-induced differentiation of primed B cells for IgG1/IgE synthesis and to prevent endocytosis or processing of the antigen by the antigen-specific B cells strongly suggest that TsF would suppress the immune responses through similar mechanisms. One might speculate that association of TsF with antigen-primed B cells through GIF receptors will facilitate the interaction of the TCR portion of TsF to antigenic peptide-MHC complex on the B cells, and that the dual interaction will make the immunosuppressive effect of GIF selective for the homologous antigen system. Formation of TCRα-GIF conjugate is unique for Ts cells. Although inactive GIF peptide is being synthesized in Th cells, cross-linking of CD3 on Th cells induced neither the translocation of GIF peptide through ER nor the formation of TCRα-GIF conjugate. However, Th cells could produce TCRα- GIF conjugate if one transfects the Pro-CT-GIF cDNA into Th cells for translocation of the **fusion protein** into ER and facilitates the recipient cells to form bioactive GIF. In this connection, it should be noted that culture of Th cells in the presence of bioactive GIF, which facilitate the Th cells to form their own bioactive GIF, confer the Th cells the capacity to form antigen-specific GIF upon antigenic stimulation (Ohno et al., 1990). Since the antigen-primed Th cells express high affinity receptors for bioactive GIF, we speculate that binding bioactive GIF to the receptors on Th cells triggers translocation of cytosolic GIF into subcellular organelles for posttranslational modifications and lets them produce bioactive GIF. Although the antigen-specific GIF formed by the GIF-treated Th cells has not been biochemically identified, we suspect that TCRα-GIF conjugate is being formed by the cells upon antigenic stimulation. If this is the case, Ts cells are simply a phenotype of Th cells. The idea is in agreement with the findings by Kuchroo et al. (1990) that no difference between Ts1 and Th cells was found in terms of cell surface markers and interleukin production, and with the observations of Green et al. (1987) that representative Th clone D10G4.1 could produce TsF1 upon stimulation with antigen-pulsed UV-irradiated macrophages. Our experiments provided evidence that OVA-specific GIF, which is an effector type TsF, consists of the 55-kDa TCRα chain-GIF conjugate and the TCR/β chain. The 55-kDa GIF itself did not retain in the OVA-coupled Sepharose. Similarly, OVA-specific TsF characterized by Chen et al. (1994) consists of TCRα and TCRβ chains. In contrast, NP-specific TsF1 could be

formed by the TCRβ expression variants of Ts1 cells, indicating that TCRβ chain is not required for the formation of the antigen-specific TsF1. It is rather surprising that the TCRα chain or its derivative has affinity for NP- hapten. However, the possibility remains that the TsF1 is a homodimer of TCRα chain derivative. Evidence was presented that NP-specific TsF1 has GIF bioactivity and shares a common antigenic determinant with the 13-kDa GIF (Steele et al., 1989). These findings suggest that the TsF1 is a 55-kDa TCRα chain-GIF conjugate, or its homodimer. It is obvious, however, that our hypothesis on the nature of TsF may not be applied to the **KLH**-specific TsF and GAT-specific TsF, whose relationship to TCR is unknown. It should be noted that these TsFs were obtained from cell extract and could not be released from the Ts cells. In contrast, antigen-specific GIF appears to be released from Ts cells through constitutive secretory pathway and does not exist in the cytosol. It has been believed that the role of TsF1 in the Ts cascade is to generate Ts2 cells. Evidence was presented by Aoki et al. (1993) that the 'factor presenting cells' in the cascade are macrophage-like adherent cells bearing I-J determinant (I-J interaction molecules) (cf., Fig. 1). If TsF1 is a TCRα-GIF conjugate, absence of GIF receptors on macrophage/monocyte cell line cells or dendritic cells raised the question: how can TsF1 associate with the 'factor-presenting cells'? The possibility exists that the TsFs involved in DTH are conjugates of TCR chain(s) with an unknown cytokine other than GIF, and the cytokine has affinity for macrophages. However, another possibility is that GIF receptors may be expressed on a subset of accessory cells. Kawasaki et al. (1986) indicated that usual macrophage cell line cells, such as P388D1, cannot be the target cells of NP-specific TsF. Using cloned macrophage hybridoma cells, Kuchroo et al. (1989) indicated that 2-h incubation of one of the hybridoma clones or splenic adherent cells with TsF was sufficient for pulsing the factor- presenting cells and that the TsF-pulsed macrophages activated precursors of Ts3. This experimental procedure indicates that TsF2 actually bound to the factor-presenting cells and conferred the cells with the capacity to activate Ts cascade. These findings suggested the possibility that the factor- presenting cells or a subset of macrophages might bear high affinity receptors for TsF/GIF. It is well known that macrophages synthesize inactive GIF. One may speculate that bioactive GIF/TsF may trigger the factor- presenting cells to form their own bioactive GIF in a similar manner as that observed in Th cells. Biochemical mechanisms involved in the Ts cascade is unknown. We speculate, however, that GIF-GIF receptor interaction, which may induced translocation of inactive cytosolic GIF peptide into subcellular organelles for the production of bioactive GIF, is involved in the cellular interactions in the suppressor pathway. Many problems on Ts and TsF remain to be solved. Further studies are required for understanding Ts cascade and the basis of I-J restriction. The most fundamental question to be answered is that whether the TsF plays essential roles in immuneregulation. Nevertheless, we believe that series of research carried out by many investigators, including ourselves, provided convincing evidence for the presence of T cell- derived antigen-specific factors.

L4 ANSWER 14 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN

1999:18390 Document No. 130:181231 Expression of recombinant mouse sperm protein sp56 and assessment of its potential for use as an antigen in an immunocontraceptive vaccine. Hardy, Christopher M.; Mobbs, Karen J. (Vertebrate Biocontrol Cooperative Research Centre, CSIRO Wildlife and Ecology, Canberra, ACT 2601, Australia). Molecular Reproduction and Development, 52(2), 216-224 (English) 1999. CODEN: MREDEE. ISSN: 1040-452X. Publisher: Wiley-Liss, Inc..

AB Recombinant mouse sp56 protein was produced for testing as an antigen in an immunocontraceptive vaccine. The coding sequence for the mature sp56 protein was cloned into the bacterial expression system pFLAG using a PCR-based method on mouse testis cDNA. Polyclonal antisera were raised in mice against affinity purified recombinant sp56 **fusion**

protein (sp56FLAG) or an artificial sp56 peptide fused to a carrier protein (**KLH**) and shown to cross-react to a protein band of 75 kDa in detergent exts. of mouse sperm by Western immunoblot anal. under reducing conditions. The antisera to sp56FLAG also immunolocalized over the entire acrosome of mouse sperm. Female BALB/c mice were immunized i.p. with sp56FLAG in a fertility trial with 20 μg sp56FLAG in Freund's Complete Adjuvant and boosted three to five times with 20 μg sp56FLAG in Freund's Incomplete Adjuvant. Litter sizes of sp56FLAG-treated mice were significantly smaller than control-treated animals after five boosts.

L4 ANSWER 15 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
1999:27997 Document No. 130:78451 Method for the immuno-isolation of a biological material using an ELISA that contains a **fusion protein** with six lysines and a polymer that ensures the enhanced immobilization. Mallet, Francois; Delair, Thierry; Ladaviere, Catherine; Novelli-Rousseau, Armelle; Charles, Marie-Helene (Bio Merieux, Fr.). PCT Int. Appl. WO 9859241 A1 19981230, 36 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (French). CODEN: PIXXD2. APPLICATION: WO 1998-FR1299 19980619. PRIORITY: FR 1997-8055 19970620.

AB The invention concerns a method for the immuno-recognition and/or binding of a biol. material from a sample using a system that consists of an organic component that ensures the enhanced immobilization of the capturing protein. The protein is genetically engineered; it contains a six lysine or lysine derivative unit that enables the enhanced immobilization to the organic component; the protein is also labeled with biotin. The organic component is a polymer, biol. or synthetic, e.g. polylysine, maleic anhydride-Me vinyl ether (MAMVE) copolymer; the organic component is supported by a carrier. Thus the recombinant protein RH24K containing the PG(K)6SVDESL sequence at the amino terminal was expressed, purified, immobilized onto MAMVE and used in ELISA assays. Proteins containing the (K)6SVDESL sequence were recognized after immunization; these were the P400 peptide (C(K)6SVDESL), the protein RH24K containing beside the polylysine sequence a polyhistidine sequence at the carboxyl terminal for chelate purification The ELISA was also used for the determination of the IG2D4 **antibody** from ascites.

L4 ANSWER 16 OF 21 MEDLINE on STN DUPLICATE 3
1998355601. PubMed ID: 9692852. Rabbit monoclonal Fab derived from a phage display library. Foti M; Granucci F; Ricciardi-Castagnoli P; Spreafico A; Ackermann M; Suter M. (CNR Cellular and Molecular Pharmacology Center, Milano, Italy.. mariaf@farma.csfic.mi.cnr.it) . Journal of immunological methods, (1998 Apr 15) 213 (2) 201-12. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Rabbit monoclonal **antibodies** (RmAb) are not routinely obtained by eukaryotic cell fusion techniques. Therefore, we have applied phage display technology to produce a recombinant rabbit Fab molecule directed against the **KLH** model antigen. The Fab fragments selected from the rabbit phage display library were subcloned in an expression vector to permit the production of a **fusion protein** comprising a dimer of bacterial alkaline phosphatase (phoA). This **fusion protein** was directly produced into the periplasmic space of Escherichia coli. We show that a crude extract containing these conjugates can be used in a direct enzyme immunoassay, as exemplified in the case of the **KLH** antigen.

L4 ANSWER 17 OF 21 EMBASE

on STN DUPLICATE 4

1999030329 EMBASE Induction of anti-HMW-MAA immunity by anti-idiotypic MAB MK2-23-IL-2 **fusion protein**. Xuemin C.; Danya L.; Baoyan W.. L. Danya, Department of Hematology, Second Affiliated Hospital, Xi'an Medical University, Xi'an 710061, China. Journal of Xi'an Medical University, English Edition 10/2 (125-132) 1998.
Refs: 11.
ISSN: 1000-923X. CODEN: JXMUEC. Pub. Country: China. Language: English.
Summary Language: English.

AB The fusion of IL-2 to anti-id mAb MK2-23 which bears the internal image of human high molecular weight melanoma-associated antigen (HMW-MAA) was used to immunize a group of BALB/c mice. The results demonstrate that the **fusion protein** is capable of eliciting significant levels of specific Abs against HMW-MAA without use of carrier proteins or adjuvants. The **fusion protein** with or without conjugating to **KLH** appear to be unique in its ability to induce high titers of anti-anti-idAbs, whereas the mAb MK2-23 in combination with IL-2 did not. We conclude that anti-id MK2-23-IL-2 **fusion protein** is a potential immunogen eliciting significant anti-tumor immunity.

L4 ANSWER 18 OF 21 MEDLINE on STN

97149913. PubMed ID: 8996707. Monoclonal **antibody** against bovine Lactoferricin and its epitopic site. Shimazaki K; Nam M S; Harakawa S; Tanaka T; Omata Y; Saito A; Kumura H; Mikawa K; Igarashi I; Suzuki N. (Dairy Science Laboratory, Animal Science Department, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.) Journal of veterinary medical science / the Japanese Society of Veterinary Science, (1996 Dec) 58 (12) 1227-9. Journal code: 9105360. ISSN: 0916-7250. Pub. country: Japan. Language: English.

AB Bovine lactoferricin (LFcin B) is a strong antimicrobial peptide derived from N-lobe of lactoferrin. To study the immunochemical and structural properties of LFcin B, monoclonal **antibody** (mAb) was prepared and the amino acid sequence concerning with the binding to mAb has been identified. Mice injected with LFcin B showed no production of **antibody** specific to this peptide, whereas those with LFcin B-**KLH** conjugate produced anti-LFcin B **antibodies**. None of the mAb reacted with bovine lactoferrin C-lobe, human lactoferrin or LFcin H. By the reactivity of the mAb against the peptides synthesized on cellulose membranes using SPOTs and against chemically modified derivatives of LFcin B, the antigenic determinant of LFcin B was identified to be the sequence of "QWR".

L4 ANSWER 19 OF 21 MEDLINE on STN DUPLICATE 5

91130571. PubMed ID: 1993465. Trypanosoma cruzi: cellular and **antibody** response against the parasite in mice immunized with a 19-amino acid synthetic peptide. Bua J; Bontempi E J; Levin M; Orn A; Velasco D; Moreno M; Levi-Yeyati P; Engstrom A; Segura E L; Ruiz A M. (Instituto Nacional de Diagnostico e Investigacion de la Enfermedad de Chagas Dr. Mario Fatala Chaben, Buenos Aires, Argentina.) Experimental parasitology, (1991 Jan) 72 (1) 54-62. Journal code: 0370713. ISSN: 0014-4894. Pub. country: United States. Language: English.

AB Several monoclonal **antibodies** were prepared against the flagellar fraction of Trypanosoma cruzi epimastigotes (Tulahuen strain, stock Tul 2). One of them, FCH-F8-4, has previously shown biologic activity against the parasite (complement-mediated lysis and neutralization of the trypomastigote infectivity). Immunopurified antigens using this monoclonal **antibody** elicited a protective immune response in mice. Two recombinant cDNA clones were detected with this anti-flagellar fraction monoclonal **antibody** on a lambda gt11 expression library prepared from T. cruzi epimastigote mRNA. The insert of one of these cDNA clones, lambda(FCH-F8-4)1 (150 bp) coded for a 19-amino acid peptide (PAFLGCSSRFSGSFSGVEP). This insert hybridized with

a 5.0-kb mRNA from epimastigotes. The beta-galactosidase **fusion protein** was produced in lysogenic bacteria. The monoclonal **antibody** recognized the epitope present in the **fusion protein** after western blotting of the crude lysate. A synthetic peptide (SP4) containing the complete sequence of lambda(FCH-F8-4)1 was constructed on solid phase. This peptide was able to inhibit the ELISA reactivity (in a range from 13 to 52%) of flagellar fraction immunized mouse sera and when administered (coupled to **KLH** or alone) to BALB/c mice with Bordetella pertussis as adjuvant, it induced a humoral and cellular immune response which was detected by ELISA, immunofluorescence, blotting, and DTH reactions against T. cruzi antigens. The immune response obtained indicates that this synthetic peptide resembles the parasite antigen conformation and could be useful for diagnosis purposes or be able to elicit immunoprotection against T. cruzi infection.

L4 ANSWER 20 OF 21 MEDLINE on STN

91194974. PubMed ID: 1707507. Clonal repertoire analysis of murine B cells specific for repeat sequence antigens of Plasmodium falciparum. Venn A J; Anders R F; Pike B L; Shortman K. (Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia.) Parasite immunology, (1990 Nov) 12 (6) 605-21. Journal code: 7910948. ISSN: 0141-9838. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Clonal analysis of the murine B-cell repertoire has been used to investigate the possible role of tandem repeat sequence epitopes of Plasmodium falciparum in immune evasion. A limiting dilution culture system was used whereby murine spleen cells were stimulated with the B-cell mitogen lipopolysaccharide (LPS) in the presence of 3T3 fibroblast filler cells. One in three B cells were shown to produce clones secreting immunoglobulin measurable by an ELISA. The frequency of **antibody** forming cell precursors (AFCp) specific for the 3' repeat epitopes of the ring injected erythrocyte surface antigen (RESA) was estimated in non-primed mice and found to be low. However, an accurate frequency determination was not possible using this method since the detection of the few positive cultures was found to depend on the presence of more than one AFCp or its products. Limiting dilution analysis was used to assess the frequency and repertoire of splenic AFCp at various times after immunization with a synthetic peptide of the RESA 3' repeat epitope (8 x 4-mer), presented in various ways. There was no marked increase in LPS-responsive AFCp specific for this antigen at the level of either IgM or IgG secretion. This was in marked contrast to the **antibody** response in vivo, where moderate IgG **antibody** titres, normally indicative of a secondary response, were seen in the serum of the same mice used for AFCp assay. This discrepancy between serum titre and AFCp frequency following immunization was not apparent with a non-malarial antigen, keyhole limpet haemocyanin (**KLH**). It was concluded that the LPS-stimulated limiting dilution culture system was not registering RESA-specific memory AFCp. These results raise the possibility that the malarial antigens are deficient in memory B-cell generation, or that secondary responses to these determinants may arise from a distinct B-cell progenitor which is non-responsive to LPS in vitro.

L4 ANSWER 21 OF 21 MEDLINE on STN DUPLICATE 6

88009610. PubMed ID: 3655607. Immunization against an inhibin subunit produced by recombinant DNA techniques results in increased ovulation rate in sheep. Forage R G; Brown R W; Oliver K J; Atrache B T; Devine P L; Hudson G C; Goss N H; Bertram K C; Tolstoshev P; Robertson D M; +. (Biotechnology Australia Pty. Ltd., East Roseville, New South Wales.) Journal of endocrinology, (1987 Aug) 114 (2) R1-4. Journal code: 0375363. ISSN: 0022-0795. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Seven Merino-Border Leicester cross-bred ewes were immunized with a purified **fusion protein**, produced by recombinant DNA methods, of the alpha subunit of bovine inhibin. Four animals were

immunized with the **fusion protein** alone and three with a conjugate made by coupling the **fusion protein** to keyhole limpet haemocyanin (**KLH**) using glutaraldehyde. Each animal received four injections of the **fusion protein** over 93 days. The animals were synchronized using progestagen sponges and subjected to laparoscopy for the determination of ovulation rates in two consecutive cycles (days 115 and 135). The immunized animals had overall mean ovulation rates for each cycle of 3.4 and 3.4 which was significantly (P less than 0.001) above the rates of 1.1 and 1.4 determined for the controls, which had either received no treatment (n = 5) or had been immunized with 300 micrograms **KLH** (n = 4). Analysis of antisera taken on day 115 showed significant **fusion protein** **antibodies** and iodinated inhibin-binding capacity in the test but not control groups. Furthermore, antisera to the **fusion** **protein** in four out of seven ewes neutralized the inhibin bioactivity of ovine follicular fluid in an in-vitro bioassay. These data demonstrate that neutralization of inhibin can be effected by immunization with bovine inhibin alpha subunit and that such immunization results in increased ovulation rates as predicted from the biological role of inhibin as a suppressor of FSH.

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=> d his

     (FILE 'HOME' ENTERED AT 16:55:57 ON 04 MAY 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 16:56:32 ON
     04 MAY 2004
L1       158774 S FUSION PROTEIN
L2        42887 S L1 AND ANTIBODY
L3           35 S L2 AND KLH
L4           21 DUP REMOVE L3 (14 DUPLICATES REMOVED)

=> s l2 and Fc fusion
L5          914 L2 AND FC FUSION

=> s l5 and complement
L6           31 L5 AND COMPLEMENT

=> dup remove l6
PROCESSING COMPLETED FOR L6
L7           15 DUP REMOVE L6 (16 DUPLICATES REMOVED)

=> d l7 1-15 cbib abs

L7   ANSWER 1 OF 15  CAPLUS  COPYRIGHT 2004 ACS on STN
2004:80848   Document No. 140:144692  IgG Fc/HIV-gp120/C3d fusion
     protein. Haynes, Barton F.; Montefiori, David C. (Duke
     University, USA). PCT Int. Appl. WO 2004009785 A2 20040129, 23 pp.
     DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ,
     CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE,
     GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
     LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL,
     PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG,
     US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU; RW: AT, BE,
     BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT,
     LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  (English).  CODEN:
     PIXXD2.  APPLICATION: WO 2003-US22917 20030723.  PRIORITY: US
     2002-PV397605 20020723.
AB   The present invention relates, in general, to an immunogen and, in
     particular, to an immunogen for inducing antibodies that
     neutralize a wide spectrum of HIV primary isolates.  The invention also
     relates to a method of inducing anti-HIV antibodies using same.
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In one example, the immunogen comprises a **fusion protein** of **complement** C3d, HIV envelope protein, and IgG Fc fragment.

L7 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:656809 Document No. 139:196279 Chimeric proteins comprising autoantigen epitope and effector molecule epitope for preventing and treating autoimmune diseases. Zocher, Marcel; Dreier, Torsten; Baeuerle, Patrick (Micromet A.-G., Germany). PCT Int. Appl. WO 2003068822 A2 20030821, 141 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-EP1389 20030212. PRIORITY: EP 2002-3332 20020213.

AB The present invention relates to a (poly)peptide construct consisting of at least two domains of at least two pluralities of domains wherein one of said domains or pluralities of domains comprises a de-immunized autoreactive antigen or (a) fragment(s) thereof specifically recognized by the Ig receptors of an autoreactive B-cells and wherein a/the further domain or plurality of domains comprises an effector mol. capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes. Preferably, said (poly)peptide construct consisting of at least two domains comprises a de-immunized autoreactive antigen or (a) fragment which is MOG or (a) fragment(s) thereof and a second domain comprising an effector mol. is an anti-CD3 receptor or an Fc-part of an Ig. The invention also relates to compns. comprising the compds. of the invention. Described is also the use of the afore-mentioned (poly)peptide construct and further compds. for the preparation of a pharmaceutical composition for the treatment and/or prevention of an autoimmune disease. In addition, the present invention relates to method for treating, ameliorating and/or preventing of an autoimmune disease. Thus, MOG-CD3, MOG-Fc, mutated MOG-Fc and AchR-**Fc fusion proteins** were prepared for eliminating autoreactive B cells.

L7 ANSWER 3 OF 15 MEDLINE on STN
2003277702. PubMed ID: 12806611. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. Van den Brande Jan M H; Braat Henri; van den Brink Gijs R; Versteeg Henri H; Bauer Christiaan A; Hoedemaeker Inge; van Montfrans Catherine; Hommes Daan W; Peppelenbosch Maikel P; van Deventer Sander J H. (Laboratory for Experimental Internal Medicine, G2-133, Academic Medical Center, Meibergdreef 9, NL-1105 AZ Amsterdam, The Netherlands.. j.vandenbrande@amc.uva.nl) . Gastroenterology, (2003 Jun) 124 (7) 1774-85. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB BACKGROUND & AIMS: Steroid-refractory Crohn's disease responds to therapy with the chimeric anti-tumor necrosis factor (TNF)-alpha **antibody** infliximab. Etanercept, a recombinant TNF receptor/immunoglobulin G **fusion protein**, is highly effective in rheumatoid arthritis but not in Crohn's disease. Because both infliximab and etanercept are TNF-alpha-neutralizing drugs, we investigated the differences in TNF-alpha-neutralizing capacity and human lymphocyte binding and apoptosis-inducing capacity of both molecules. METHODS: We used a nuclear factor kappaB reporter assay and a cytotoxicity bioassay to study TNF-alpha neutralization by infliximab and etanercept. Lymphocyte binding and apoptosis-inducing capacity was investigated using fluorescence-activated cell sorter analysis, annexin V staining, and cleaved caspase-3 immunoblotting using mixed lymphocyte reaction-stimulated peripheral blood lymphocytes (PBL) from healthy

volunteers and lamina propria T cells from patients with Crohn's disease. RESULTS: Both infliximab and etanercept neutralized TNF-alpha effectively. Infliximab bound to activated PBL and lamina propria T cells, whereas binding of etanercept was equal to a nonspecific control **antibody**. Infliximab but not etanercept induced peripheral and lamina propria lymphocyte apoptosis when compared with a control **antibody**. Infliximab activated caspase 3 in a time-dependent manner, whereas etanercept did not. CONCLUSIONS: Although both infliximab and etanercept showed powerful TNF-alpha neutralization, only infliximab was able to bind to PBL and lamina propria T cells and subsequently to induce apoptosis of activated lymphocytes. These data may provide a biological basis for the difference in efficacy of the 2 TNF-alpha-neutralizing drugs.

L7 ANSWER 4 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003096155 EMBASE BAFF: A novel therapeutic target for autoimmunity. Kalled S.L.. S.L. Kalled, Biogen Inc., 12 Cambridge Center, Cambridge, MA 02142, United States. Susan_Kalled@biogen.com. Current Opinion in Investigational Drugs 3/7 (1005-1010) 1 Jul 2002.
Refs: 42.
ISSN: 1472-4472. CODEN: CIDREE. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Autoimmunity results from a break in self-tolerance involving humoral and/or cell-mediated immune mechanisms. One pathological consequence of a failure in central and/or peripheral tolerance is the generation of autoantibodies and subsequent formation of **complement**-fixing immune complexes that contribute to tissue damage. Prevailing pharmacological strategies for treating autoimmune diseases involve the use of broad-acting immunosuppressants that with long term use have associated toxicities. The current drive in drug development is towards therapies that target a specific biological pathway or pathogenic cell population. Recent discovery of the BAFF-mediated B-cell survival pathway provides a unique opportunity for developing focused intervention for autoreactive B-cell function.

L7 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

2002:601108 Document No. 137:351232 Effects of CTLA4-Fc on glomerular injury in humorally-mediated glomerulonephritis in BALB/c mice. Kitching, A. R.; Huang, X. R.; Ruth, A.-J.; Tipping, P. G.; Holdsworth, S. R. (Centre for Inflammatory Diseases, Monash Medical Centre, Monash University Department of Medicine, Clayton, Victoria, Australia). Clinical and Experimental Immunology, 128(3), 429-435 (English) 2002. CODEN: CEXIAL. ISSN: 0009-9104. Publisher: Blackwell Science Ltd..

AB The effect of cytotoxic T lymphocyte-associated mol. 4-Ig **fusion protein** (CTLA4-Fc) on humorally-mediated glomerulonephritis was studied in accelerated anti-glomerular basement membrane (anti-GBM) glomerulonephritis induced in BALB/c mice. This strain of mice develops **antibody** and **complement** dependent glomerulonephritis under this protocol. Sensitized BALB/c mice developed high levels of circulating autologous **antibody** titers, intense glomerular deposition of mouse Ig and **complement**, significant proteinuria, renal impairment, significant glomerular necrosis and a minor component of crescent formation 10 days after challenge with a nephritogenic antigen (sheep anti-GBM globulin). Early treatment during the primary immune response, or continuous treatment throughout the disease with CTLA4-Fc, significantly suppressed mouse anti-sheep globulin **antibody** titers in serum, and Ig and **complement** deposition in glomeruli. The degree of glomerular necrosis was improved and proteinuria was reduced, particularly in the earlier stages of disease. Late treatment by CTLA4-Fc starting one day after challenge with sheep anti-mouse GBM did not affect **antibody** production and did not attenuate glomerulonephritis. The low level of crescent formation found in BALB/c mice developing glomerulonephritis was not prevented by the administration

of CTLA4-Fc. These results demonstrate that CTLA4-Fc is of benefit in this model of glomerulonephritis by its capacity to attenuate **antibody** production, without affecting the minor degree of cell-mediated glomerular injury.

L7 ANSWER 6 OF 15 MEDLINE on STN DUPLICATE 1
2002459345. PubMed ID: 12218370. Monoclonal **antibodies** against the C(epsilon)mX domain of human membrane-bound IgE and their potential use for targeting IgE-expressing B cells. Chen Huan Yuan; Liu Fu-Tong; Hou Charlie M H; Huang Janice S W; Sharma Bhavya Bhavna; Chang Tse Wen. (Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan, ROC.) International archives of allergy and immunology, (2002 Aug) 128 (4) 315-24. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: IgE mediates immediate-type hypersensitivity reactions responsible for various allergic symptoms. It is secreted by IgE-producing plasma cells, which differentiate from B cells expressing membrane-bound IgE (mIgE) on their surface. The epsilon-chain of human mIgE contains a membrane-anchoring peptide and an extra 52-amino-acid (a.a.)-long domain (referred to as C(epsilon)mX) between the membrane anchor and the CH4 domain. OBJECTIVE: The study was designed to evaluate the effects of C(epsilon)mX-specific monoclonal **antibodies** (mAbs) to target IgE-expressing B cells and decrease IgE production. METHODS: A C(epsilon)mX-containing IgG1.**Fc fusion protein** was produced in CHO cells and used to immunize mice; five hybridoma clones secreting C(epsilon)mX-specific mAbs were obtained. RESULTS: Characterization of the mAbs using ELISA, immunoprecipitation, and immunoblotting methods showed that they could bind to both native and denatured forms of C(epsilon)mX. The mAbs exhibited mutual inhibition of binding to mIgE. Epitope mapping using synthetic peptides revealed that all five mAbs recognize the same epitope, RADWPGPP, located near the C-terminus of C(epsilon)mX. Binding of one of the mAbs to mIgE on SKO-007 cells induced the cross-linking of mIgE molecules on the cell surface, resulting in their patching and capping. In vitro functional analysis revealed that mAbs are able to cause **complement**-mediated cytotoxicity on transfectants expressing the Fc portion of mIgE. CONCLUSION: We have prepared several human mIgE-specific mAbs. The potential of the mAbs on targeting mIgE+ B cells was demonstrated by CDC analysis.

L7 ANSWER 7 OF 15 MEDLINE on STN DUPLICATE 2
2002410752. PubMed ID: 12165074. Coupling **complement** regulators to immunoglobulin domains generates effective anti-**complement** reagents with extended half-life in vivo. Harris C L; Williams A S; Linton S M; Morgan B P. (Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff, UK.. HarrisCL@cardiff.ac.uk) . Clinical and experimental immunology, (2002 Aug) 129 (2) 198-207. Journal code: 0057202. ISSN: 0009-9104. Pub. country: England: United Kingdom. Language: English.

AB **Complement** activation and subsequent generation of inflammatory molecules and membrane attack complex contributes to the pathology of a number of inflammatory and degenerative diseases, including arthritis, glomerulonephritis and demyelination. Agents that specifically inhibit **complement** activation might prove beneficial in the treatment of these diseases. Soluble recombinant forms of the naturally occurring membrane **complement** regulatory proteins (CRP) have been exploited for this purpose. We have undertaken to design better therapeutics based on CRP. Here we describe the generation of soluble, recombinant CRP comprising rat decay accelerating factor (DAF) or rat CD59 expressed as **Fc fusion proteins**, **antibody**-like molecules comprising two CRP moieties in place of the **antibody** Fab arms (CRP-Ig). Reagents bearing DAF on each

arm (DAF-Ig), CD59 on each arm (CD59-Ig) and a hybrid reagent containing both DAF and CD59 were generated. All three reagents inhibited C activation in vitro. Compared with soluble CRP lacking Fc domains, activity was reduced, but was fully restored by enzymatic release of the regulator from the Ig moiety, implicating steric constraints in reducing functional activity. In vivo studies showed that DAF-Ig, when compared to soluble DAF, had a much extended half-life in the circulation in rats and concomitantly caused a sustained reduction in plasma **complement** activity. When given intra-articularly to rats in a model of arthritis, DAF-Ig significantly reduced severity of disease. The data demonstrate the potential of CRP-Ig as reagents for sustained therapy of inflammatory disorders, including arthritis, but emphasize the need for careful design of **fusion proteins** to retain function.

L7 ANSWER 8 OF 15 MEDLINE on STN

2002052048. PubMed ID: 11777558. Treatment of experimental autoimmune myasthenia gravis with recombinant human tumor necrosis factor receptor Fc protein. Christadoss Premkumar; Goluszko Elzbieta. (Department of Microbiology and Immunology, University of Texas Medical Branch, 301 University Boulevard, 3.142 MRB, Galveston, TX 77555-1070, USA.. pchrista@utmb.edu) . Journal of neuroimmunology, (2002 Jan) 122 (1-2) 186-90. Journal code: 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB Lymphotoxin-alpha (TNF-beta) and TNF receptor p55 gene knockout mice are resistant to the development of **antibody** and **complement** mediated experimental autoimmune myasthenia gravis (EAMG), suggesting a possible role of TNF in mediating EAMG. Therefore, we tested the hypothesis that blocking the functional interaction of TNF with their receptors by soluble recombinant human TNFR:Fc would suppress the ongoing clinical EAMG. Recombinant human TNFR:Fc administered daily for 2 weeks to C57BL6 mice with ongoing clinical EAMG significantly improved clinical EAMG when compared with placebo-treated mice. A clinical trial of selected myasthenia gravis patients with recombinant human TNFR:Fc could be attempted.

L7 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

2001:798070 Document No. 135:343299 Bispecific opsonins. Himawan, Jeff (Elusys Therapeutics, Inc., USA). PCT Int. Appl. WO 2001080883 A1 20011101, 93 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US13161 20010424. PRIORITY: US 2000-PV199903 20000426; US 2000-PV244812 20001101.

AB The author discloses bispecific mols. that are characterized by having a first domain which binds an antigen and a second domain which binds the C3b-like receptor (known as **complement** receptor 1 (CR1) or CD35 in primates). In one example, a bispecific **antibody** is prepared that targets both IgE and the C3b receptor.

L7 ANSWER 10 OF 15 MEDLINE on STN DUPLICATE 3

2002126912. PubMed ID: 11809933. Multimerization of a chimeric anti-CD20 single-chain Fv-**Fc fusion protein** is mediated through variable domain exchange. Wu A M; Tan G J; Sherman M A; Clarke P; Olafsen T; Forman S J; Raubitschek A A. (Department of Molecular Biology, Divison of Biology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010, USA.) Protein engineering, (2001 Dec) 14 (12) 1025-33. Journal code: 8801484. ISSN: 0269-2139. Pub. country: England: United Kingdom. Language: English.

AB A series of single-chain anti-CD20 **antibodies** was produced by fusing single-chain Fv (scFv) with human IgG1 hinge and Fc regions, designated scFv-Fc. The initial scFv-Fc construct was assembled using an 18 amino acid (aa) linker between the **antibody** light- and heavy-chain variable regions, with the Cys residue in the upper hinge region (Kabat 233) mutagenized to Ser. Anti-CD20 scFv-Fc retained specific binding to CD20-positive cells and was active in mediating **complement**-dependent cytolysis. Size-exclusion HPLC analysis revealed that the purified scFv-Fc included multimeric as well as monomeric components. Variant scFv-Fcs were constructed incorporating four different hinges between the scFv and Fc regions, or three different linkers in the scFv domain. All formed multimers, with the highest level of multimerization found in the scFv-Fc with the shortest linker (8 aa). Elimination of an unusual salt bridge between residues L38 and H89 in the V(L)-V(H) domain interface failed to reduce the formation of higher order forms. Structural analysis of the scFv-Fc constructed with 18 or 8 aa linkers by pepsin or papain cleavage suggested the proteins contained a form in which scFv units had cross-paired to form a 'diabody'. Thus, domain exchange or cross-pairing appears to be the basis of the observed multimerization.

L7 ANSWER 11 OF 15 MEDLINE on STN DUPLICATE 4

2001048384. PubMed ID: 10934210. Molecular and cellular properties of the rat AA4 antigen, a C-type lectin-like receptor with structural homology to thrombomodulin. Dean Y D; McGreal E P; Akatsu H; Gasque P. (Brain Inflammation and Immunity Group, Medical Biochemistry Department, University of Wales College of Medicine, Cardiff, CF144XN, United Kingdom.) Journal of biological chemistry, (2000 Nov 3) 275 (44) 34382-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The murine fetal stem cell marker AA4 has recently been cloned and is known to be the homolog of the human phagocytic C1q receptor involved in host defense. We herein report the molecular cloning and the cellular expression pattern of the rat AA4 antigen. Modular architecture analysis indicated that the rat AA4 is a member of C-type lectin-like family and, interestingly, displays similar domain composition and organization to thrombomodulin. Northern blot and reverse transcriptase-polymerase chain reaction analyses indicated that rat AA4 was encoded by a single transcript of 7 kilobases expressed constitutively in all tissues. In situ hybridization showed that AA4 was expressed predominantly by pneumocytes and vascular endothelial cells. Using an affinity purified polyclonal **antibody** raised against a rat AA4-**Fc fusion protein**, AA4 was identified as a glycosylated protein of 100 kDa expressed by endothelial cells > platelets > NK cells and monocytes (ED1+ cells). The staining was associated to the cell surface and intracytoplasmic vesicles. Conversely, erythrocytes, T and B lymphocytes, neutrophils, and macrophages (ED2+ cells) were consistently negative for AA4. As expected, the macrophage cell line NR8383 expressed weak levels of AA4. Taken together, our results support the idea that AA4/C1qRp is involved in some cell-cell interactions.

L7 ANSWER 12 OF 15 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2000:779904 The Genuine Article (R) Number: 362BC. Intercellular adhesion molecule 1 and beta 2 integrins in C1q-stimulated superoxide production by human neutrophils - An example of a general regulatory mechanism governing acute inflammation. Tyagi S; NicholsonWeller A; Barbashov S F; Tas S W; Klickstein L B (Reprint). BRIGHAM & WOMENS HOSP, DIV RHEUMATOL ALLERGY & IMMUNOL, SMITH BLDG ROOM 614, 1 JIMMY FUND WAY, BOSTON, MA 02115 (Reprint); BRIGHAM & WOMENS HOSP, DIV RHEUMATOL ALLERGY & IMMUNOL, BOSTON, MA 02115; HARVARD UNIV, SCH MED, BOSTON, MA; BETH ISRAEL DEACONESS MED CTR, BOSTON, MA 02215. ARTHRITIS AND RHEUMATISM (OCT 2000) Vol. 43, No. 10, pp. 2248-2259. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621. ISSN: 0004-3591. Pub. country: USA.

Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Objective, To investigate the role of intercellular adhesion molecule 1 (ICAM-1) and beta 2 integrins in the production of superoxide (O-2(-)) by C1q-stimulated human polymorphonuclear leukocytes (PMN).

Methods. PMN were pretreated with F(ab')(2) fragments of monoclonal **antibodies** (mAb) that blocked or did not block beta 2 integrin-mediated adhesion. The cells were added to wells coated with C1q, and the production of O-2(-) was monitored kinetically as a color change due to reduction of cytochrome c, In some experiments, C1q was co-immobilized with purified ICAM-1,

Results. Blocking mAb to the shared beta 2 integrin subunit, CD18, completely inhibited the O-2(-) response triggered by immobilized C1q, while blocking mAb to the alpha subunits of the beta 2 integrins each partially blocked the O-2(-) response. PMN treated with C1q were found to activate the beta 2 integrins lymphocyte function-associated antigen 1 and CR3 for binding to ICAM-1. Co-immobilization of ICAM-1 with C1q cooperatively triggered O-2(-) production by PMN,

Conclusion. beta 2 integrin binding to an ICAM provided an essential costimulatory signal for O-2(-) production triggered by C1q in PMN, Our findings suggest a model for PMN activation in which 2 stimuli are required for O-2(-) production: a first signal that also activates PMN beta 2 integrins, followed by a second, beta 2 integrin-mediated signal, which occurs physiologically upon PMN binding to ICAM-1, The requirement for this dual signal for PMN generation of O-2(-) would serve as a regulatory mechanism to limit the production of O-2(-) to a tissue environment where C1q, or some other stimulus, is colocalized with stromal cells bearing upregulated ICAM-1, This mechanism may explain why all tissues can express ICAM-1 and may explain in part why inhibitors of tumor necrosis factor alpha, a major physiologic stimulus of ICAM-1 up-regulation, are potent antiinflammatory agents.

L7 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 5

1999421861. PubMed ID: 10491008. IL-2 receptor-targeted cytolytic IL-2/**Fc fusion protein** treatment blocks diabetogenic autoimmunity in nonobese diabetic mice. Zheng X X; Steele A W; Hancock W W; Kawamoto K; Li X C; Nickerson P W; Li Y; Tian Y; Strom T B. (Department of Medicine, Division of Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA.) Journal of immunology (Baltimore, Md. : 1950), (1999 Oct 1) 163 (7) 4041-8. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB High affinity IL-2R5 is present on recently activated but not on resting or memory T cells. Selective targeting of T cells bearing high affinity IL-2R is an attractive therapy for many T cell-dependent cytopathic disease processes. A variety of rodent mAbs directed against the alpha-chain of the IL-2R, as well as IL-2 fusion toxins, have been used in animals and humans to achieve selective immunosuppression. Here we report on the development of a novel IL-2R targeting agent, a cytolytic chimeric IL-2/**Fc fusion protein**. This immunoligand binds specifically and with high affinity to IL-2R and is structurally capable of recruiting host Ab-dependent cell-mediated cytotoxicity and **complement**-dependent cytotoxicity activities. The Ig component ensures an extended circulating t1/2 of 25 h following systemic administration. To subsequently explore the mechanisms of the antidiabetogenic effects of IL-2/Fc, we have mutated the FcR binding and **complement** C1q binding (Fc-/-) domains of the Fc fragment to render the Fc unable to direct Ab-dependent cell-mediated cytotoxicity and **complement**-dependent cytotoxicity activities. In a model of passive transfer of diabetes in nonobese diabetic mice, lytic IL-2/Fc, but not nonlytic IL-2/Fc-/-, exhibited striking antidiabetogenic effects. Together with the negligible potential of IL-2/Fc for immunogenicity, this finding forecasts that cytolytic IL-2/Fc may offer a new therapeutic

approach for selective targeting of auto and alloimmune T cells.

L7 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1998:13862 Document No. 128:57452 Use of chimeric vaccinia virus **complement** control proteins to inhibit **complement**, and use in xenotransplantation and treatment of **complement**-mediated disorders. Rosengard, Ariella M.; Ahearn, Joseph M., Jr.; Sanfilippo, Alfred P.; Baldwin, William M., III (Johns Hopkins University School of Medicine, USA). PCT Int. Appl. WO 9747321 A1 19971218, 36 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US10310 19970613. PRIORITY: US 1996-19920 19960614; US 1996-19801 19960614.

AB Disclosed are chimeric proteins that are useful for inhibiting **complement**. The chimeric protein, termed VCPFc, is a **fusion protein** in which (i) an Ig Fc region is fused to (ii) a polypeptide that comprises a portion of a vaccinia virus **complement** control protein which binds **complement** components C4b and C3b, but not iC3b rosettes. This protein can be used in xenograft transplantation methods (e.g., by treating the donor mammal or organ) and in methods for treating **complement**-mediated disorders (e.g., inflammation) generally. In a second chimeric protein, a transmembrane anchoring domain is fused to a polypeptide that comprises a portion of a vaccinia virus **complement** control protein which binds **complement** components C4b and C3b, but not iC3b rosettes. The transmembrane anchoring domain can be, for example, short consensus regions 3 through 15 of human **complement** receptor 2 protein. Expression of the transmembrane-anchored **fusion protein** in a transgenic animal provides a well-suited organ donor for xenograft transplantation.

L7 ANSWER 15 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 6
95148807 EMBASE Document No.: 1995148807. Administration of noncytolytic IL-10/Fc in murine models of lipopolysaccharide-induced septic shock and allogeneic islet transplantation. Xin Xiao Zheng; Steele A.W.; Nickerson P.W.; Steurer W.; Steiger J.; Strom T.B.. Division of Immunology, Department of Medicine, Beth Israel Hospital, 330 Brookline Avenue,Boston, MA 02215, United States. Journal of Immunology 154/10 (5590-5600) 1995. ISSN: 0022-1767. CODEN: JOIMA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Numerous studies have suggested the potential application of IL-10 as an anti-inflammatory and as an antirejection agent. Unfortunately, cytokines have short circulating t1/2 We developed a murine IL-10/Fcγ2a immunoligand that possesses the biologic functions of IL-10 and the long circulating t1/2 in vivo, characteristic of Igs. We mutated the Fcγ2a fragment to render the immunoligand ineffective in directing Ab-dependent cell-mediated cytotoxicity and **complement**-directed cytolysis (noncytolytic IL-10/Fc (IL-10/Fc2-)). In terms of IL-10 activity, IL-10/Fc2- was as effective as rIL-10 mole per mole in preventing lethal septic shock, but the immunoligand had a prolonged period of efficacy in accord with its extended circulating half-life. Contrary to expectations, IL-10/Fc2- treatment tended to accelerate the destruction of islet cell allografts and increase the levels of granzyme B gene expression in local draining lymph nodes. These data suggest that the enhanced cytotoxic activity of allograft-destroying CTLs may contribute to the accelerated allograft rejection. Finally, our studies suggest that a noncytolytic IL-10/**Fc fusion protein** provides a useful tool to study the biologic effects of IL-10 in vivo and

may provide a useful agent for the prevention and treatment of septic shock.

=> s antiidiotypic antibody
L8 3474 ANTIIDIOTYPIC ANTIBODY

=> s l8 and fusion
L9 137 L8 AND FUSION

=> s l9 and C3d
L10 1 L9 AND C3D

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L10 ANSWER 1 OF 1 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
1998:363378 The Genuine Article (R) Number: ZL948. Enhanced molecular mimicry of CEA using photoaffinity crosslinked **C3d** peptide. Lou D Y; Kohler H (Reprint). IMMPHERON INC, LEXINGTON, KY 40509 (Reprint); IMMPHERON INC, LEXINGTON, KY 40509; UNIV KENTUCKY, DEPT MICROBIOL & IMMUNOL, LEXINGTON, KY 40536. NATURE BIOTECHNOLOGY (MAY 1998) Vol. 16, No. 5, pp. 458-462. Publisher: NATURE PUBLISHING CO. 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707. ISSN: 1087-0156. Pub. country: USA. Language: English .

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Antigen mimicry of using anti-idiotypic antibodies for use as cancer vaccines has been disappointing due to the weak immunogenicity of immunoglobulin variable domains. To enhance the immunogenicity of an anti-idiotype vaccine we incorporated a molecular adjuvant peptide into the antibody. The peptide is derived from the **C3d** region known to bind CR2 receptors on B-cells. A photoreactive peptide is synthesized that affinity-labels a single site in the antibody variable domain. The molecular adjuvant peptide is crosslinked to the anti-idiotype mimetic by chemical means without modifying other sites on the antibody. The **C3d**-conjugated anti-idiotype antibody induces a strong idiotype and antigen-specific response in mice.

=> s l9 and "38C13"
L11 0 L9 AND "38C13"

=> s l9 and "3H1"
L12 0 L9 AND "3H1"

=> s C3d peptide
L13 23 C3D PEPTIDE

=> s l13 and fusion
L14 3 L13 AND FUSION

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PROCESSING COMPLETED FOR L14
L15 3 DUP REMOVE L14 (0 DUPLICATES REMOVED)

=> d l15 1-3 cbib abs

L15 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
2003:435073 Document No. 139:21018 **Fusion** proteins of biologically active peptides and antibodies as vaccines. Kohler, Heinz (USA). U.S. Pat. Appl. Publ. US 2003103984 A1 20030605, 10 pp., Cont.-in-part of U.S. 6,238,667. (English). CODEN: USXXCO. APPLICATION: US 2001-865281 20010529. PRIORITY: US 1998-70907 19980504.

AB The invention provides a **fusion** protein made up of (1) an

antibody and (2) a peptide having a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and homophilic activities wherein the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody. In the present invention that is accomplished by a method comprising the steps of creating a **fusion** gene including a nucleic acid sequence encoding an antibody and a nucleic acid sequence encoding the peptide, wherein the nucleic acid sequence encoding the peptide is located inside the nucleic acid sequence encoding the antibody at a site wherein, when the **fusion** is expressed, the **fusion** protein created thereby comprises the antibody and the peptide, wherein the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody. The invention also provides a composition and a pharmaceutical composition comprising a **fusion** protein of a peptide with a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and homophilic activities and an antibody. An example is presented of enhancing the activity of an anti-idiotypic vaccine consisting of an anti-idiotypic antibody (murine anti-idiotypic antibody 3H1 which mimics the carcinoembryonic antigen) via crosslinking it with a peptide derived from the complement C3d region 1217-1232.

L15 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
1999:216944 Document No. 130:236462 Method of affinity crosslinking biologically active, immunogenic peptides to antibodies. Kohler, Heinz (USA). PCT Int. Appl. WO 9914244 A1 19990325, 33 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US19710 19980918. PRIORITY: US 1997-59515 19970919.

AB A method of affinity crosslinking a peptide to an antibody by photo-chemical activating an azido compound in a peptide comprising said azido compound; adding an antibody to the photochem. activated peptide; and allowing the photochem. activated peptide and the antibody to react. The azido compound has an affinity for a hydrophobic structure in the variable domain of the antibody which binds to nucleotides or nucleosides, binding the peptide into a native binding pocket of the Ig (Ig) structure of an antibody. The site of crosslinking is located away from the antigen binding site in the Fv domain avoiding the compromise of antigen recognition. A composition of a peptide cross-linked to an antibody is also disclosed. Thus, anti-idiotype vaccines were prepared by crosslinking 3H1, an anti-idiotype antibody that mimics carcinoembryonic antigen, or 38C13, an anti-idiotype antibody of B lymphoma, with a **C3d peptide** (i.e. KNRWEDPGKQLYNVEA) to enhance antigen presentation.

L15 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
1998:363378 The Genuine Article (R) Number: ZL948. Enhanced molecular mimicry of CEA using photoaffinity crosslinked **C3d peptide**.
Lou D Y; Kohler H (Reprint). IMMPHERON INC, LEXINGTON, KY 40509 (Reprint); IMMPHERON INC, LEXINGTON, KY 40509; UNIV KENTUCKY, DEPT MICROBIOL & IMMUNOL, LEXINGTON, KY 40536. NATURE BIOTECHNOLOGY (MAY 1998) Vol. 16, No. 5, pp. 458-462. Publisher: NATURE PUBLISHING CO. 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707. ISSN: 1087-0156. Pub. country: USA. Language: English

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Antigen mimicry of using anti-idiotypic antibodies for use as cancer vaccines has been disappointing due to the weak immunogenicity of immunoglobulin variable domains. To enhance the immunogenicity of an anti-idiotype vaccine we incorporated a molecular adjuvant peptide into

the antibody. The peptide is derived from the C3d region known to bind CR2 receptors on B-cells. A photoreactive peptide is synthesized that affinity-labels a single site in the antibody variable domain. The molecular adjuvant peptide is crosslinked to the anti-idiotype mimetic by chemical means without modifying other sites on the antibody. The C3d-conjugated anti-idiotype antibody induces a strong idiotype and antigen-specific response in mice.

=> s membrane transport peptide
L16 18 MEMBRANE TRANSPORT PEPTIDE

=> dup remove l16
PROCESSING COMPLETED FOR L16
L17 14 DUP REMOVE L16 (4 DUPLICATES REMOVED)

=> d l17 1-14 cbib abs

L17 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2003:818235 Document No. 139:322283 Methods for production and use of mammalian complementarity determining region mimetibodies for diagnosis and therapy of human diseases. Heavner, George A.; Knight, David M.; Scallon, Bernard J.; Ghrayeb, John (Centocor, Inc., USA). PCT Int. Appl. WO 2003084477 A2 20031016, 97 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US9139 20030324. PRIORITY: US 2002-PV368791 20020329.
AB This invention pertains to methods for production and use of mammalian complementarity determining region (CDR) mimetibodies for diagnosis and therapy of human diseases. Genetic engineering, expression, and purification of human mimetibodies containing Ig fragments (CDR, variable, framework and/or constant region) as well as a ligand binding domain are disclosed in this invention. Peptides that mimic the activity of EPO, TPO, growth hormones, G-CSF, GM-CSF, IL-1ra, leptin, CTLA4, TRAIL, TGF-α and TGF-β are the focus of this genetic engineering. The aim of the invention is use of the purified recombinant proteins for diagnosis or treatment of anemia, immune or autoimmune disease, cancer, or infectious diseases. At the time of publication, claimed sequence nos. 997 to 1109 were missing, and claimed sequence nos. 984 to 996 were not clearly identified.

L17 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2003:610601 Document No. 139:145561 Transport peptides and uses for deliverying drugs to target cells. Giordano, Frank J.; Sessa, William C. (Yale University, USA). PCT Int. Appl. WO 2003064614 A2 20030807, 32 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US2715 20030130. PRIORITY: US 2002-PV352745 20020130.
AB The invention describes isolated transport peptides, which cross the cell membrane of a cell and/or home to a target cell. The invention also describes a transport complex in which a transport peptide is linked to a

cargo moiety to be delivered into/to a cell. Methods are disclosed describing delivery of a transport complex into and/or to a cell. Vectors and host cells comprising transport peptides and transport complexes are also described, as well as pharmaceutical compns. including transport complexes of the present invention.

L17 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2003:377047 Document No. 138:379195 Peptide nucleic acids against early genes of human papillomaviruses and conjugates containing them to inhibit viral infection. Braun, Klaus; Waldeck, Waldemar; Pipkorn, Ruediger; Braun, Isabell; Debus, Juergen (Deutsches Krebsforschungszentrum Stiftung Des Oeffentlichen Rechts, Germany). PCT Int. Appl. WO 2003040365 A2 20030515, 34 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (German). CODEN: PIXXD2. APPLICATION: WO 2002-DE4153 20021108. PRIORITY: DE 2001-10154831 20011108.

AB The invention concerns peptide-nucleic acid (PNA) conjugates, either singly or in mixts., that inhibit the expression of human papillomavirus genes (HPV) E6 or E7. Preferably, the peptide nucleic acid conjugates are directed against HPV16 or HPV18 for treatment of cervical carcinoma. The PNA is conjugated with a peptide that stimulates cell uptake and a nuclear localization signal. The components of the conjugate may be connected by labile disulfide bridges. Use of these conjugates to induce apoptosis in HeLa cells, a cervical cancer line, is demonstrated.

L17 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2003:435073 Document No. 139:21018 Fusion proteins of biologically active peptides and antibodies as vaccines. Kohler, Heinz (USA). U.S. Pat. Appl. Publ. US 2003103984 A1 20030605, 10 pp., Cont.-in-part of U.S. 6,238,667. (English). CODEN: USXXCO. APPLICATION: US 2001-865281 20010529. PRIORITY: US 1998-70907 19980504.

AB The invention provides a fusion protein made up of (1) an antibody and (2) a peptide having a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and homophilic activities wherein the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody. In the present invention that is accomplished by a method comprising the steps of creating a fusion gene including a nucleic acid sequence encoding an antibody and a nucleic acid sequence encoding the peptide, wherein the nucleic acid sequence encoding the peptide is located inside the nucleic acid sequence encoding the antibody at a site wherein, when the fusion is expressed, the fusion protein created thereby comprises the antibody and the peptide, wherein the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody. The invention also provides a composition and a pharmaceutical composition comprising a fusion protein of a peptide with a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and homophilic activities and an antibody. An example is presented of enhancing the activity of an anti-idiotypic vaccine consisting of an anti-idiotypic antibody (murine anti-idiotypic antibody 3H1 which mimics the carcinoembryonic antigen) via crosslinking it with a peptide derived from the complement C3d region 1217-1232.

L17 ANSWER 5 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2004:152079 Document No.: PREV200400147729. Targeting the genomic DNA of the BCR-ABL gene as a new molecular treatment strategy for chronic myelogenous leukemia. Topaly, Julian [Reprint Author]; Radujkovic, Aleksandar; Braun,

Klaus; Debus, Juergen; Zeller, W. Jens; Ho, Anthony D. [Reprint Author]; Fruehauf, Stefan [Reprint Author]. Internal Medicine V, University of Heidelberg, Heidelberg, Germany. Blood, (November 16 2003) Vol. 102, No. 11, pp. 651a. print.
Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Classical molecular inhibitors of the BCR-ABL oncogene target the aberrant protein or mRNA. Quiescent leukemic progenitors with low expression of BCR-ABL shown an increased resistance to these agents. We investigated the potential of peptide nucleic acids (PNAs) to bind to genomic DNA and to inhibitor proliferation of BCR-ABL positive leukemic cells. In PNAs the backbone is replaced by an uncharged flexible polyamide resulting in increased affinity to DNA and resistance to endogenous proteases and nucleases. We used a modular transporter bearing a cellular **membrane transport peptide** and, as a cargo, a PNA covalently linked to a nuclear localisation signal (NLS). Transport peptide and PNA are connected via N-terminal activated cysteine to form cleavable disulphide bonds. Internalization and subsequent delivery of PNA to the nucleus was verified in living and fixed cells by confocal laser scanning microscopy and fluorescence correlation spectroscopy (FCS). Double-labelling experiments indicate the cytoplasmic cleavage of the two modules and the effective nuclear import of the chromophore-tagged cargo. A non-degradable linker between transport module and cargo as well as a construct without NLS did not enable nuclear PNA import under the described experimental conditions. FCS measurements revealed that most of the PNAs delivered into the cytoplasm by the modular transporter are anchored or encapsulated, indicating that intracellular transport of these compounds is not governed by molecular diffusion. PNAs targeting genomic intron sequences of ABL, BCR, BCR-ABL fusion region of K562 cells, and random sequences were synthesized. A MTT assay with K562 cells showed a concentration-dependent cell growth inhibition by the modular transporter containing PNAs targeting the ABL or BCR introns (e.g. 0.46% mean survival at 10 muM, 5.9% at 5 muM, 61.7% at 2.5 muM, 95.2% at 1 muM with a PNA targeting the ABL intron after 72 hours of exposure; similar data were obtained with a PNA targeting the BCR intron) while random PNA sequences were not inhibitory. The modular transporter containing a PNA targeting the BCR-ABL fusion region of K562 cells was even more effective (55+-22% survival at 1 muM). Our data prove the feasibility of the proposed PNA-based treatment strategy for BCR-ABL-positive chronic myelogenous leukemia.

L17 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2003:813606 Document No. 140:144321 MTS-conjugated-antiactive caspase 3 antibodies inhibit actinomycin D-induced apoptosis. Zhao, Y.; Brown, T. L.; Kohler, H.; Mueller, S. (Immpheron Inc., Lexington, KY, 40503, USA). Apoptosis, 8(6), 631-637 (English) 2003. CODEN: APOPFN. ISSN: 1360-8185. Publisher: Kluwer Academic Publishers.

AB Caspase 3 is critically involved in the pathway of apoptosis. The authors have conjugated a MTS-transport-peptide to monoclonal and polyclonal anti-caspase-3 antibodies to suppress Actinomycin D-induced apoptosis in human lymphoma T cells. The advantage of using trans-membrane antibodies compared to conventional apoptosis inhibitors is their specific target recognition in the living cell and their lower toxicity compared to conventional apoptosis inhibitors. The authors could show that a MTS-transport-peptide modified monoclonal anti-caspase-3 antibody reduces Actinomycin D induced apoptosis, as shown by DNA ladder electrophoresis and cell death ELISA. These results indicate that antibodies have a therapeutic potential to inhibit apoptosis in a variety of diseases.

L17 ANSWER 7 OF 14 MEDLINE on STN DUPLICATE 1
2002308648. PubMed ID: 12051833. A biological transporter for the delivery of peptide nucleic acids (PNAs) to the nuclear compartment of living

cells. Braun Klaus; Peschke Peter; Pipkorn Rudiger; Lampel Stefan; Wachsmuth Malte; Waldeck Waldemar; Friedrich Eckhard; Debus Jurgen. (Division of Radiooncology, Klinische Kooperationseinheit Strahlentherapeutische Onkologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany.) Journal of molecular biology, (2002 Apr 26) 318 (2) 237-43. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB To facilitate nuclear delivery of biomolecules we describe the synthesis of a modular transporter bearing a cellular **membrane transport peptide** (pAntp) and, as a cargo, a 16-mer peptide nucleic acid (PNA) covalently linked to a nuclear localisation signal (NLS[SV40-T]). Transport peptide and PNA are connected via N-terminal activated cysteine to form cleavable disulphide bonds. Internalization and subsequent delivery of PNA to the nucleus was verified in living and fixed cells by confocal laser scanning microscopy (CLSM) and fluorescence correlation spectroscopy (FCS). Double-labelling experiments indicate the cytoplasmic cleavage of the two modules and the effective nuclear import of the chromophore-tagged cargo. A non-degradable linker between transport module and cargo as well as a construct without NLS did not enable nuclear PNA import under the described experimental conditions. FCS-measurements revealed that most of the PNAs delivered into the cytoplasm by the modular transporter are anchored or encapsulated, indicating that intracellular transport of these compounds is not governed by molecular diffusion. Our results clearly demonstrate efficient compartment-directed transport using a synthetic, non-toxic modular transporter in living cells.

L17 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

2003:457787 Document No. 140:141122 Transportans. Pooga, Margus; Hallbrink, Mattias; Langel, Ulo (Estonian Biocentre, Tartu, Estonia). Cell-Penetrating Peptides, 53-70. Editor(s): Langel, Uelo. CRC Press LLC: Boca Raton, Fla. ISBN: 0-8493-1141-1 (English) 2002. CODEN: 69EAO3.

AB A review on the discovery, properties, and application of the cell-penetrating peptide transportan and its analogs. Transportan has been successfully utilized for the cellular delivery of peptides, proteins, and peptide nucleic acid.

L17 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

2003:457785 Document No. 140:141121 The Tat-derived cell-penetrating peptide. Vives, Eric; Lebleu, Bernard (Institut de Genetique Moleculaire, Universite de Montpellier, Montpellier, Fr.). Cell-Penetrating Peptides, 3-21. Editor(s): Langel, Uelo. CRC Press LLC: Boca Raton, Fla. ISBN: 0-8493-1141-1 (English) 2002. CODEN: 69EAO3.

AB A review discusses the application of the Tat-derived cell-penetrating peptide as a vehicle for the cellular delivery of chemical conjugated biomols. Tat-mediated delivery of a wide collection of cargo mols. from medium-size material (oligonucleotides or peptides) to large particles (phages or liposomes) has been achieved. Such type of delivery has been successfully applied to cell types difficult to transfect by usual methodologies, e.g., monocyte or macrophage progenitors.

L17 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1994:498930 Document No. 121:98930 Characterization of bacterial **membrane transport peptides** using toxophoric agent, N-OH-Ala. Hong, Nam-joo (Yeungnam Univ., Gyungsan City, 712-749, S. Korea). Pept.: Biol. Chem., Proc. Chin. Pept. Symp., Meeting Date 1992, 153-5. Editor(s): Du, Yu-cang; Tam, James P.; Zhang, You-shang. ESCOM: Leiden, Neth. (English) 1993. CODEN: 59YOAI.

AB The author describes the use of N-hydroxyalanine (N-OH-Ala) as the warhead component of peptides and the in vitro activity of these peptides against E. coli.

L17 ANSWER 11 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1990:297710 Document No.: PREV199039015891; BR39:15891. BIOPHYSICAL MODEL APPROACHES TO MECHANISTIC TRANSEPITHELIAL STUDIES OF PEPTIDES. HO N F H [Reprint author]; DAY J S; BARSUHN C L; BURTON P S; RAUB T J. DRUG DELIVERY SYST RES, PHARM RES DEV DIV, UPJOHN CO, KALAMAZOO, MICH 49001, USA. (1990) pp. 3-24. ANDERSON, J. M., S. W. KIM AND K. KNUTSON (ED.). ADVANCES IN DRUG DELIVERY SYSTEMS, VOL. 4; INTERNATIONAL SYMPOSIUM ON RECENT ADVANCES IN DRUG DELIVERY SYSTEMS, SALT LAKE CITY, UTAH, USA, FEBRUARY 21-24, 1989. X+359P. ELSEVIER SCIENCE PUBLISHERS B.V.: AMSTERDAM, NETHERLANDS; NEW YORK, NEW YORK, USA. ILLUS. Publisher: Series: Advances in Drug Delivery Systems.
ISBN: 0-444-88225-1. Language: ENGLISH.

L17 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1980:616712 Document No. 93:216712 Transmembrane transport of small peptides. Matthews, D. M.; Payne, J. W. (Dep. Exp. Chem. Pathol., Westminster Hosp., London, UK). Current Topics in Membranes and Transport, 14, 331-425 (English) 1980. CODEN: CTMTA2. ISSN: 0070-2161.
AB A review with many refs.

L17 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1980:616736 Document No. 93:216736 The chemistry of membrane-active peptides and proteins. Ovchinnikov, Yu. A. (Shemyakin Inst. Bioorgan. Chem., Moscow, USSR). Soviet Scientific Reviews, Section D: Biology Reviews, 1, 1-41 (English) 1980. CODEN: SRSRDL. ISSN: 0143-0424.
AB A review with 68 refs. on peptides and proteins involved in transport by biol. membranes.

L17 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1970:129901 Document No. 72:129901 Phosphopeptides in the erythrocyte stroma. Ledig, M.; Waitz, R.; Hauptmann, G. R.; Mandel, Paul (Centre Neurochim., Fac. Med., Strasbourg, Fr.). Bulletin de la Societe de Chimie Biologique, 51(7-8), 1237-9 (French) 1969. CODEN: BSCIA3. ISSN: 0037-9042.
AB Phospholipids (I), phosphopeptides (II), and phosphoproteins (III) were isolated from lyophilized erythrocyte stroma. The II and III levels of erythrocyte stroma were similar to those of the mitochondrial and microsomal membranes of rat liver, whereas those in the myelin sheath were much higher. However, values in the total rat liver were .apprx.50% lower, thus indicatin g the preferential localization of II in the tissue membrane. In contrast to II the values for III in the natural membranes were mu ch less elevated. The II/III ratio was .apprx.2. Results furthermore indicated that the I/II ratio was a good criterio n for determining the abundance of II in the tissues. II was postulated to be active in membrane transport.

=> s peptide
L18 1532831 PEPTIDE

=> s l18 and homophilic activity
L19 2 L18 AND HOMOPHILIC ACTIVITY

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PROCESSING COMPLETED FOR L19
L20 2 DUP REMOVE L19 (0 DUPLICATES REMOVED)

=> d l20 1-2 cbib abs

L20 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
2003:435073 Document No. 139:21018 Fusion proteins of biologically active **peptides** and antibodies as vaccines. Kohler, Heinz (USA). U.S. Pat. Appl. Publ. US 2003103984 A1 20030605, 10 pp., Cont.-in-part of U.S.

6,238,667. (English). CODEN: USXXCO. APPLICATION: US 2001-865281 20010529. PRIORITY: US 1998-70907 19980504.

AB The invention provides a fusion protein made up of (1) an antibody and (2) a **peptide** having a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and **homophilic activities** wherein the **peptide** is connected to the antibody at a site that does not interfere with antigen binding of the antibody. In the present invention that is accomplished by a method comprising the steps of creating a fusion gene including a nucleic acid sequence encoding an antibody and a nucleic acid sequence encoding the **peptide**, wherein the nucleic acid sequence encoding the **peptide** is located inside the nucleic acid sequence encoding the antibody at a site wherein, when the fusion is expressed, the fusion protein created thereby comprises the antibody and the **peptide**, wherein the **peptide** is connected to the antibody at a site that does not interfere with antigen binding of the antibody. The invention also provides a composition and a pharmaceutical composition comprising a fusion protein of a **peptide** with a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and **homophilic activities** and an antibody. An example is presented of enhancing the activity of an anti-idiotypic vaccine consisting of an anti-idiotypic antibody (murine anti-idiotypic antibody 3H1 which mimics the carcinoembryonic antigen) via crosslinking it with a **peptide** derived from the complement C3d region 1217-1232.

L20 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

2002:927546 Document No. 138:23668 Fusion proteins of antibodies and biologically active **peptides** with increased therapeutic value and their preparation. Kohler, Heinz; Morgan, Charles (Immpheron, Inc., USA; Innexus Corporation). PCT Int. Appl. WO 2002097041 A2 20021205, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US16651 20020529. PRIORITY: US 2001-865281 20010529.

AB Fusion proteins of antibodies and **peptides** having a biol. activity such as immunostimulation, membrane transport and **homophilic activities** in which the **peptide** is connected to the antibody at a site that does not interfere with antigen binding of the antibody are described for therapeutic use. The protein is manufactured by expression of a cloned gene in which the **peptide** coding sequence is inserted into the antibody gene at a site where the **peptide** will not interfere with the antigen binding properties of the antibody. The **peptide** may be flanked by loop-forming or conformation-conferring sequences. The use of a **peptide** derived from complement C3 to improve the antigenicity of a monoclonal antibody to carcinoembryonic antigen is demonstrated.

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=> s Fc fusion protein
L21        2138 FC FUSION PROTEIN

=> s l21 and receptor
L22        1678 L21 AND RECEPTOR

=> s l22 and cellular
L23         108 L22 AND CELLULAR
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=> s l23 and tumor cell
   4 FILES SEARCHED...
L24   6 L23 AND TUMOR CELL

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PROCESSING COMPLETED FOR L24
L25   4 DUP REMOVE L24 (2 DUPLICATES REMOVED)

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L25 ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1
2004168628. PubMed ID: 15002034. Carcinoembryonic antigen-immunoglobulin **Fc fusion protein** (CEA-Fc) for identification and activation of anti-CEA immunoglobulin-T-cell **receptor** -modified T cells, representative of a new class of Ig fusion proteins. Ma Qiangzhong; DeMarte Luisa; Wang Yawen; Stanners Clifford Paul; Junghans Richard Paul. (1Biotherapeutics Development Lab, Division of Hematology-Oncology, Beth Israel Deaconess Medical Center and Harvard Institute of Human Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.) Cancer gene therapy, (2004 Apr) 11 (4) 297-306. Journal code: 9432230. ISSN: 0929-1903. Pub. country: England: United Kingdom. Language: English.

AB Chimeric immunoglobulin-T-cell **receptor** (IgTCR)-modified T cells ("designer T cells") kill **tumor cells** based on antibody-redirected recognition of tumor-associated antigen. Anti-carcinoembryonic antigen (CEA) designer T cells have been prepared and applied in adoptive **cellular** immunotherapy regimens for CEA-positive cancers. A CEA-immunoglobulin Fc (CEA-**Fc**) **fusion protein** was created from the A3B3 region of CEA and the Fc portion of human IgG for the purposes of activation and detection of anti-CEA designer T cells. CEA-Fc was expressed at high yield in CHO cells and purified to homogeneity in a single step on a protein A affinity column. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed that CEA-Fc formed disulfide-linked dimers with a molecular weight of about 170 kDa and a monomer size of 85kDa. The A3B3 CEA component of the CEA-Fc bound to anti-CEA monoclonal antibody MN-14, as well as to the single-chain Fv (sFv) derived from this antibody that was expressed in the IgTCR on the surface of designer T cells. The Fc portion of CEA-Fc was recognized by anti-human IgG Fc antibody and bound by human monocyte Fc **receptors**. CEA-Fc activated the anti-CEA designer T cells as plate-bound or monocyte-bound form but not as soluble form, as measured by CD69 expression and T-cell proliferation. Our results indicate that the CEA-**Fc** **fusion protein** can be used to detect the expression of the anti-CEA IgTCR chimeric **receptors** on the modified T cells, as well as to serve as an antigen to activate the anti-CEA IgTCR modified T cells. CEA-Fc is the prototype for a new class of antigen-Fc molecules that may significantly augment the analytic and therapeutic goals of adoptive designer T-cell immunotherapies.Cancer Gene Therapy (2004) 11, 297-306. doi:10.1038/sj.cgt.7700685 Published online 27 February 2004

L25 ANSWER 2 OF 4 MEDLINE on STN
2001395137. PubMed ID: 11294894. Layilin, a novel integral membrane protein, is a hyaluronan **receptor**. Bono P; Rubin K; Higgins J M; Hynes R O. (Howard Hughes Medical Institute, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139, USA.) Molecular biology of the cell, (2001 Apr) 12 (4) 891-900. Journal code: 9201390. ISSN: 1059-1524. Pub. country: United States. Language: English.

AB The actin cytoskeleton plays a significant role in changes of cell shape and motility, and interactions between the actin filaments and the cell membrane are crucial for a variety of **cellular** processes. Several adaptor proteins, including talin, maintain the

cytoskeleton-membrane linkage by binding to integral membrane proteins and to the cytoskeleton. Layilin, a recently characterized transmembrane protein with homology to C-type lectins, is a membrane-binding site for talin in peripheral ruffles of spreading cells. To facilitate studies of layilin's function, we have generated a layilin-**Fc** **fusion protein** comprising the extracellular part of layilin joined to human immunoglobulin G heavy chain and used this chimera to identify layilin ligands. Here, we demonstrate that layilin-**Fc** **fusion protein** binds to hyaluronan immobilized to Sepharose. Microtiter plate-binding assays, coprecipitation experiments, and staining of sections predigested with different glycosaminoglycan-degrading enzymes and cell adhesion assays all revealed that layilin binds specifically to hyaluronan but not to other tested glycosaminoglycans. Layilin's ability to bind hyaluronan, a ubiquitous extracellular matrix component, reveals an interesting parallel between layilin and CD44, because both can bind to cytoskeleton-membrane linker proteins through their cytoplasmic domains and to hyaluronan through their extracellular domains. This parallelism suggests a role for layilin in cell adhesion and motility.

L25 ANSWER 3 OF 4 MEDLINE on STN

1998184542. PubMed ID: 9525630. A novel membrane protein is a mouse mammary tumor virus **receptor**. Golovkina T V; Dzuris J; van den Hoogen B; Jaffe A B; Wright P C; Cofer S M; Ross S R. (Department of Microbiology/Cancer Center, University of Pennsylvania, Philadelphia 19104-6142, USA.) Journal of virology, (1998 Apr) 72 (4) 3066-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mouse mammary tumor virus (MMTV) infects a number of different cell types, including mammary gland and lymphoid cells, in vivo. To identify the **cellular receptor** for this virus, a mouse cDNA expression library was transfected into Cos-7 monkey kidney cells, and those transfected cells able to bind virus were selected by using antibody against the virus's cell surface envelope protein, gp52. One clone isolated from a library prepared from newborn thymus RNA, called MTVR, was able to confer virus binding to both monkey and human cells; this binding was blocked by anti-MTVR antibody. Moreover, transfection of MTVR into CV1 cells rendered them susceptible to infection by a murine leukemia virus-based retrovirus vector pseudotyped with the MMTV envelope protein. An epitope-tagged MTVR cofractionated with **cellular** membranes. Coimmunoprecipitation of the MMTV envelope protein and a MTVR-rabbit **Fc fusion protein** showed that these two proteins bound to each other. The MTVR sequence clone is unique, shows no homology to known membrane proteins, and is transcribed in many tissues.

L25 ANSWER 4 OF 4 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

96:805007 The Genuine Article (R) Number: VP736. HUMAN EOSINOPHILS EXPRESS FUNCTIONAL CD30 LIGAND AND STIMULATE PROLIFERATION OF A HODGKINS-DISEASE CELL-LINE. PINTO A (Reprint); ALDINUCCI D; GLOGHINI A; ZAGONEL V; DEGAN M; IMPROTA S; JUZBASIC S; TODESCO M; PERIN V; GATTEI V; HERRMANN F; GRUSS H J; CARBONE A. IRCCS, CTR REG RIFERIMENTO ONCOL, DEPT MED ONCOL, LEUKEMIA UNIT, VIA PEDEMONTANA OCCIDENTALE, I-33081 AVIANO, ITALY (Reprint); CTR RIFERIMENTO ONCOL, DEPT MED ONCOL, I-33081 AVIANO, ITALY; CTR RIFERIMENTO ONCOL, DEPT PATHOL, I-33081 AVIANO, ITALY; UNIV ULM, MED CTR, DEPT HEMATOL & ONCOL INTERNAL MED 3, ULM, GERMANY. BLOOD (01 NOV 1996) Vol. 88, No. 9, pp. 3299-3305. ISSN: 0006-4971. Pub. country: ITALY; GERMANY. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The presence of a prominent tissue eosinophilia represents a typical histopathologic hallmark of Hodgkin's disease (HD). To evaluate the putative role of eosinophils on **tumor cell** regulation in HD, we have analyzed these cells for the functional expression of CD30 ligand (CD30L), a surface molecule able to transduce CD30-mediated

proliferation signals on Hodgkin's (H) and Reed-Sternberg (RS) cells. The results demonstrate that circulating and tissue eosinophils from normal donors and patients with HD or hypereosinophilic syndrome (HES), display CD30L mRNA and express CD30L protein, as shown by immunostaining with a specific monoclonal antibody (M80) and with a biotinylated soluble CD30-**Fc fusion protein**. The surface density of CD30L on eosinophils from HD and HES patients was remarkably higher compared with healthy donors, probably reflecting a cytokine-mediated upregulation in these pathologic conditions. Accordingly, we provide evidence that cytokines regulating eosinophils proliferation and activation, ie, interleukin-5 (IL-5), IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF), are able to enhance the **cellular** density of CD30L on purified eosinophils from normal subjects. Finally, we show that native CD30L on human eosinophils is a functionally active surface structure able to transduce proliferative signals on CD30(+) target cells, including cultured H-RS cells. Our data suggest that eosinophils may not merely represent innocent bystanders, but rather act as important elements in the pathology of HD by contributing to the deregulated network of CD30/CD30L-mediated interactive signals between H-RS cells and surrounding reactive cells. (C) 1996 by The American Society of Hematology.

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L28 ANSWER 1 OF 53 MEDLINE on STN DUPLICATE 1
2004114226. PubMed ID: 15004154. Carcinoembryonic antigen-related **cellular** adhesion molecule 1 isoforms alternatively inhibit and costimulate human T cell function. Chen Daohong; Iijima Hideki; Nagaishi Takashi; Nakajima Atsushi; Russell Sara; Raychowdhury Raktima; Morales Victor; Rudd Christopher E; Utku Nalan; Blumberg Richard S. (Gastroenterology Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA.) Journal of immunology (Baltimore, Md. : 1950), (2004 Mar 15) 172 (6) 3535-43. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Carcinoembryonic Ag-related **cellular** adhesion molecule 1 (CEACAM1) represents a group of transmembrane protein isoforms that consist of variable numbers of extracellular Ig-like domains together with either a long cytoplasmic (cyt) tail containing two immunoreceptor tyrosine-based inhibitory motifs or a unique short cyt tail. Although CEACAM1 has been reported to be expressed on the surface of T lymphocytes upon activation, its roles in T cell regulation are controversial due to the lack of functional characterization of each individual CEACAM1 isoform. We thus cotransfected Jurkat T cells with CEACAM1 isoform-encoding constructs and an IL-2 promoter-bearing plasmid or a small interference RNA targeting src homology domain 2 containing phosphatase 1. In a luciferase reporter assay and through measurements of cytokine secretion (IL-2, IL-4, and IFN-gamma), CEACAM1 containing either a long or a short cyt tail inhibited or costimulated, respectively, TCR/CD3 complex plus CD28 mediated activation with the inhibitory functions of the long cyt tail dominating. The inhibitory function of CEACAM1, was dependent upon src homology domain 2 containing phosphatase 1

activity, required both tyrosine residues within the immunoreceptor tyrosine-based inhibitory motif domains of the cyt tail and was mediated through the mitogen-activated protein kinase pathway. CEACAM1-mediated inhibition could be functionally reconstituted by incubation of PBMC with either a CEACAM1-specific mAb or CEACAM1-**Fc fusion protein** in the presence of an allogeneic or mitogenic stimulus, respectively. These studies indicate that the long and short cyt tails of CEACAM1 serve as inhibitory and costimulatory **receptors**, respectively, in T cell regulation.

L28 ANSWER 2 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004118837 EMBASE Cadherin-related neuronal **receptor** 1 (CNR1) has cell adhesion activity with β1 integrin mediated through the RGD site of CNR1. Mutoh T.; Hamada S.; Senzaki K.; Murata Y.; Yagi T.. T. Mutoh, Lab. for Cell Culture Development, Brain Science Institute, RIKEN, Saitama 351-0198, Japan. tmuto@brain.riken.go.jp. Experimental Cell Research 294/2 (494-508) 1 Apr 2004.
Refs: 34.
ISSN: 0014-4827. CODEN: ECREAL.
Publisher Ident.: S 0014-4827(03)00632-3. Pub. Country: United States.
Language: English. Summary Language: English.

AB Cadherin-related neuronal **receptor** (CNR) proteins are a diverse set of synaptic protocadherins, but little is known about its adhesive properties. We found that overexpressed CNR1 protein localized on the cell surface of HEK293T cells and increased the calcium-dependent cell aggregation potential. However, we could not detect the strong homophilic binding activity of CNR1 EC-**Fc fusion protein** in vitro. Parental HEK293T cells adhered to Arg-Gly-Asp (RGD) motif of EC1 domain of CNR1-**Fc fusion protein**. The fusion protein that the Asp (73) of EC1 point-mutated to Glu (RGE-Fc) lost the adhesive activity. The adhesion activity of HEK293T cells to CNR1 EC-**Fc fusion protein** was completely blocked by inhibitors of integrins, including RGDS peptide and anti-β1 integrin antibodies. The increased cell-aggregative property of CNR1 transfectants was also blocked by RGDS peptides. At cell-cell junctions of the CNR1 transfectants, co-localization between CNR1 and HEK293T endogenous β1 integrin was observed. Furthermore, the spatiotemporal expression patterns of CNR and β1 integrin nearly overlapped in the molecular layer of the developing mouse cerebellum in the main stage of synaptogenesis. These results indicate that CNR1 has a heterophilic, calcium-dependent cell adhesion activity with the β1 integrin subfamily, and raise the possibility of CNR-β1 integrin association in synaptogenesis.

L28 ANSWER 3 OF 53 MEDLINE on STN DUPLICATE 2

2004168628. PubMed ID: 15002034. Carcinoembryonic antigen-immunoglobulin **Fc fusion protein** (CEA-Fc) for identification and activation of anti-CEA immunoglobulin-T-cell **receptor** -modified T cells, representative of a new class of Ig fusion proteins. Ma Qiangzhong; DeMarte Luisa; Wang Yawen; Stanners Clifford Paul; Junghans Richard Paul. (1Biotherapeutics Development Lab, Division of Hematology-Oncology, Beth Israel Deaconess Medical Center and Harvard Institute of Human Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.) Cancer gene therapy, (2004 Apr) 11 (4) 297-306. Journal code: 9432230. ISSN: 0929-1903. Pub. country: England: United Kingdom.
Language: English.

AB Chimeric immunoglobulin-T-cell **receptor** (IgTCR)-modified T cells ("designer T cells") kill tumor cells based on antibody-redirected recognition of tumor-associated antigen. Anti-carcinoembryonic antigen (CEA) designer T cells have been prepared and applied in adoptive **cellular** immunotherapy regimens for CEA-positive cancers. A CEA-immunoglobulin Fc (CEA-**Fc**) **fusion protein**

was created from the A3B3 region of CEA and the Fc portion of human IgG for the purposes of activation and detection of anti-CEA designer T cells. CEA-Fc was expressed at high yield in CHO cells and purified to homogeneity in a single step on a protein A affinity column. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed that CEA-Fc formed disulfide-linked dimers with a molecular weight of about 170 kDa and a monomer size of 85kDa. The A3B3 CEA component of the CEA-Fc bound to anti-CEA monoclonal antibody MN-14, as well as to the single-chain Fv (sFv) derived from this antibody that was expressed in the IgTCR on the surface of designer T cells. The Fc portion of CEA-Fc was recognized by anti-human IgG Fc antibody and bound by human monocyte Fc **receptors**. CEA-Fc activated the anti-CEA designer T cells as plate-bound or monocyte-bound form but not as soluble form, as measured by CD69 expression and T-cell proliferation. Our results indicate that the CEA-**Fc fusion protein** can be used to detect the expression of the anti-CEA IgTCR chimeric **receptors** on the modified T cells, as well as to serve as an antigen to activate the anti-CEA IgTCR modified T cells. CEA-Fc is the prototype for a new class of antigen-Fc molecules that may significantly augment the analytic and therapeutic goals of adoptive designer T-cell immunotherapies.Cancer Gene Therapy (2004) 11, 297-306. doi:10.1038/sj.cgt.7700685 Published online 27 February 2004

L28 ANSWER 4 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004096691 EMBASE Neutrophin effects on neuroblatoma cells: Correlation with Trk and p75NTR expression and influence of Trk **receptor** bodies.
Evangelopoulos M.E.; Kruttgen A.. M.E. Evangelopoulos, Division of Neuropathology, Institute of Pathology, University of Bern, Murtenstrasse 31, CH-3010 Bern, Switzerland. evangelo@patho.unibe.ch. Journal of Neuro-Oncology 66/1-2 (101-110) 2004.
Refs: 42.
ISSN: 0167-594X. CODEN: JNODD2. Pub. Country: United States. Language: English. Summary Language: English.

AB Neurotrophins are key signalling molecules in the development of the nervous system. They elicit diverse **cellular** responses such as proliferation, differentiation, survival and apoptosis. Neurotrophins (NTs) bind to two different classes of cell surface **receptors**, Trk **receptor** tyrosine kinases and p75NTR, both of which are expressed by neuroblastoma cells. Neurotrophin signalling via Trks was shown to promote both survival and differentiation of neuroblastoma cells in vitro. The expression of certain Trk **receptors** is considered to be a prognostic indicator. The p75NTR **receptor** is the founding member of the Fas/TNF-R family, which is best known for its function in the induction of apoptosis. Its function in neuroblastomas is thus far poorly understood. We analysed neurotrophin **receptor** (NTR) expression of neuroblastoma cells by surface biotinylation assays and applied recombinant nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5 to these cell lines assessing their survival and proliferation in long-term assays lasting 6 days. NGF increased proliferation of Neuro 2a cells, which express p75NTR but no TrkA **receptors** on their surface. On the other hand, SK-N-BE cell proliferation was decreased after NGF treatment, even though these cells also express p75NTR but no TrkA **receptors** on their surface. Interestingly, neurotrophin-scavenger proteins (TrkB-Fc and TrkC-Fc) as well as chemical blockers of Trk **receptor** signalling (K252a, Wortmannin, PD98059) slowed down the proliferation of both cell lines in medium containing serum. Taken together, our results indicate that p75NTR activation has diverse effects on neuroblastomas, depending on the specific neuroblastoma clone. In addition, our studies point towards TrkB-Fc or TrkC-Fc **receptor** bodies as useful tools to influence the survival of neuroblastoma cells.

L28 ANSWER 5 OF 53 MEDLINE on STN

2003219735. PubMed ID: 12615627. Histoplasmosis after treatment with anti-tumor necrosis factor-alpha therapy. Wood Karen L; Hage Chadi A; Knox Kenneth S; Kleiman Martin B; Sannuti Aruna; Day Richard B; Wheat L Joseph; Twigg Homer L 3rd. (Division of Pulmonary and Critical Care, Department of Medicine, Infectious Disease, Indiana University School of Medicine, Indianapolis, Indiana, USA.) American journal of respiratory and critical care medicine, (2003 May 1) 167 (9) 1279-82. Journal code: 9421642. ISSN: 1073-449X. Pub. country: United States. Language: English.

AB Anti-tumor necrosis factor-alpha (TNF-alpha) antibodies are frequently used to treat inflammatory diseases. However, these drugs also have immunosuppressive effects. We report on three patients who developed disseminated histoplasmosis on therapy with TNF-alpha inhibitors. In vitro assays were used to characterize the role of these agents in host defense against Histoplasma capsulatum. Intracellular proliferation of H. capsulatum was measured in alveolar macrophages and peripheral monocytes of normal volunteers in the presence and absence of the TNF-alpha antibody, infliximab. Both infliximab and control antibody enhanced fungal growth in monocytes and alveolar macrophages, suggesting this was a nonspecific antibody response. Despite similar intracellular fungal loads in the presence of both antibodies, lymphocyte proliferation in response to blood monocytes and alveolar macrophages infected with H. capsulatum was inhibited by the addition of physiologic doses of infliximab, whereas control antibody had no effect. The production of H. capsulatum-induced interferon-gamma and TNF-alpha was assessed in 5-day cultures containing lymphocytes and alveolar macrophages or monocytes. Interferon-gamma secretion was significantly reduced in the presence of infliximab. In summary, patients receiving anti-TNF-alpha therapy are at risk for developing disseminated histoplasmosis. This may be due to a defect in the TH1 arm of **cellular** immunity.

L28 ANSWER 6 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN

2003:605872 Document No. 140:223006 Targeting and Blocking B7 Costimulatory Molecules on Antigen-Presenting Cells Using CTLA4Ig-Conjugated Liposomes: In Vitro Characterization and in Vivo Factors Affecting Biodistribution. Park, Chung-Gyu; Thiex, Natalie W.; Lee, Kyung-Mi; Szot, Gregory L.; Bluestone, Jeffery A.; Lee, Kyung-Dall (College of Pharmacy, Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI, 48109-1065, USA). Pharmaceutical Research, 20(8), 1239-1248 (English) 2003. CODEN: PHREEB. ISSN: 0724-8741. Publisher: Kluwer Academic/Plenum Publishers.

AB CTLA4Ig, a fusion protein of CTLA-4 and Fc of Ig (Ig) heavy chain, inhibits the essential costimulatory signal for full T cell activation via blocking the interaction between CD28 and B7 mols. and renders T cell nonresponsiveness. CTLA4Ig has been used to control deleterious T cell activation in many exptl. systems. We hypothesized that by conjugating CTLA4Ig to liposomes the efficacy of CTLA4Ig could be enhanced through multivalent ligand effect, superior targetability, and modification of the fate of ligated costimulatory mols. Consistent with this hypothesis, liposome-conjugated CTLA4Ig bound to B7 and blocked their binding sites more efficiently than free CTLA4Ig, lowering the half maximal dose for B7 blocking by an order of the magnitude. These results were similar both in B7-1 expressing p815 cells and in activated macrophages. Moreover, CTLA4Ig-liposomes underwent rapid internalization upon cell surface binding through B7 mols. In allogenic mixed lymphocyte reaction assays, the CTLA4Ig-liposomes were tested to show effective inhibition of T cell proliferation. In vivo, however, when CTLA4Ig-liposomes were injected into mice, a significant fraction was localized to the reticuloendothelial system (RES), presumably because of its binding to Fc **receptors** expressed on tissue macrophages. The Fc **receptor**-mediated uptake could be alleviated by coinjection of anti-FcR monoclonal antibody. In the mouse engrafted with pancreatic islets of Langerhans underneath the capsule of one kidney, despite the increased localization in RES, enhanced

accumulation of CTLA4Ig-conjugated liposome was observed in the engrafted kidney compared to the contralateral kidney. Thus, the conjugation of CTLA4Ig to liposome could increase the efficiency of the targeting by increasing the binding avidity at **cellular** level and by increasing the concentration at the target site in in vivo system. The biodistribution and circulation time data suggested that the CTLA4Ig-liposomes could be improved upon minimizing the FcR-mediated uptake by Fc **receptor**-bearing cells. Thus, the strategy of conjugating CTLA4Ig to liposomes could be exploited for immune intervention in transplantation and autoimmune diseases for the efficient blocking of costimulation.

L28 ANSWER 7 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2004:155048 Document No.: PREV200400148513. The TNFalpha-homologue RANKL mediates doxorubicin-induced apoptosis. Mueller, Ingo [Reprint Author]; Pfister, Stefan M. [Reprint Author]; Grohs, Ulrike [Reprint Author]; Handgretinger, Rupert; Niethammer, Dietrich [Reprint Author]; Bruchelt, Gernot [Reprint Author]. Hematology and Oncology, Children's Hospital, Tuebingen, Germany. Blood, (November 16 2003) Vol. 102, No. 11, pp. 858a. print.
Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Doxorubicin induces apoptosis in a variety of cells. We investigated the expression and function of various TNFalpha-homologues and their **receptors**. RT-PCR analysis of RNA extracted from CEM cells after incubation for 12 hours in the presence of 1 muM doxorubicin. Specific primers were designed and GAPDH served as an internal standard. All investigated mRNAs (TRAIL, TRAIL-R1, the splice-forms TRICK-A and TRICK-B of TRAIL-R2, and TRAMP) were detected in CEM cells. Neither TRAIL nor one of its **receptors** showed significant variation in expression levels (data not shown). RANK was not significantly modulated in expression either, however, its cognate ligand RANKL was increased fivefold. This increase was stronger than the detected CD95L induction, which was reported earlier. The functional significance of this upregulation was demonstrated by neutralization experiments. In the presence of either one of two anti-RANKL monoclonal antibodies, doxorubicin-induced apoptosis was completely inhibited for 12 hours. The specificity was further corroborated by a RANK-**Fc fusion protein** that had similar effects. Moreover, neutralizing RANKL also prevented cytochrome c release from mitochondria. These experiments show that RANKL plays an integral role in doxorubicin-induced apoptosis that cannot be fully compensated for by CD95L. Consequently, RANKL acts independent or upstream of CD95L. Doxorubicin is known for its complex pharmacological mechanism. Therefore, we assessed the contribution of CD95L- and RANKL-induced apoptosis, respectively, by measuring the metabolic activity over time employing the MTT test. Consistent with the experiments shown above, blocking CD95L or RANKL mitigated early apoptosis-inducing effects of doxorubicin. However, neither one of the antibodies was able to completely abrogate the cytotoxicity of doxorubicin in long-term cultures. In order to explore some of the mechanistic aspects we focused on the involvement of mitochondria early in apoptosis induced by doxorubicin. Staurosporine served as a control, because it is known to disrupt the mitochondrial membrane potential as one of the first detectable **cellular** changes causing the release of cytochrome c. As expected, incubation of CEM cells with doxorubicin and staurosporine, respectively, induced cytochrome c release from the mitochondria. However, neutralizing alphaRANKL antibody inhibited this effect only in the case of doxorubicin, whereas staurosporine was still able to induce cytochrome c release within ten hours of incubation. In line with this finding, staurosporine-induced apoptosis as assessed by staining CEM cells with annexin V was not affected by the presence of this antibody either. Surprisingly, CEM cells did not become apoptotic in the presence of

rhRANKL alone. This experiment rules out non-specific binding of this TNFalpha-homologue to **receptors** like CD95 or the TRAIL, **receptor** DR5 in the concentrations under investigation. There was no change in the cell cycle analysis of CEM in the presence of 1 mug/ml rhRANKL or 3 mug/ml rhRANKL either. The incubation of CEM cells with both, RANKL and doxorubicin, however, resulted in a 2.2-fold increase of apoptosis. Therefore, RANKL is necessary but not sufficient to account for early doxorubicin-induced apoptosis in CEM cells. This finding suggests improved chemotherapeutic efficiency of the anthracyclin against susceptible malignant cells in the presence with RANKL.

L28 ANSWER 8 OF 53 MEDLINE on STN

2003366367. PubMed ID: 12900522. VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. Ishida Susumu; Usui Tomohiko; Yamashiro Kenji; Kaji Yuichi; Amano Shiro; Ogura Yuichiro; Hida Tetsuo; Oguchi Yoshihisa; Ambati Jayakrishna; Miller Joan W; Gragoudas Evangelos S; Ng Yin-Shan; D'Amore Patricia A; Shima David T; Adamis Anthony P. (Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston 02114, USA.) Journal of experimental medicine, (2003 Aug 4) 198 (3) 483-9. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Hypoxia-induced VEGF governs both physiological retinal vascular development and pathological retinal neovascularization. In the current paper, the mechanisms of physiological and pathological neovascularization are compared and contrasted. During pathological neovascularization, both the absolute and relative expression levels for VEGF164 increased to a greater degree than during physiological neovascularization. Furthermore, extensive leukocyte adhesion was observed at the leading edge of pathological, but not physiological, neovascularization. When a VEGF164-specific neutralizing aptamer was administered, it potently suppressed the leukocyte adhesion and pathological neovascularization, whereas it had little or no effect on physiological neovascularization. In parallel experiments, genetically altered VEGF164-deficient (VEGF120/188) mice exhibited no difference in physiological neovascularization when compared with wild-type (VEGF+/+) controls. In contrast, administration of a VEGFR-1/**Fc fusion protein**, which blocks all VEGF isoforms, led to significant suppression of both pathological and physiological neovascularization. In addition, the targeted inactivation of monocyte lineage cells with clodronate-liposomes led to the suppression of pathological neovascularization. Conversely, the blockade of T lymphocyte-mediated immune responses with an anti-CD2 antibody exacerbated pathological neovascularization. These data highlight important molecular and **cellular** differences between physiological and pathological retinal neovascularization. During pathological neovascularization, VEGF164 selectively induces inflammation and **cellular** immunity. These processes provide positive and negative angiogenic regulation, respectively. Together, new therapeutic approaches for selectively targeting pathological, but not physiological, retinal neovascularization are outlined.

L28 ANSWER 9 OF 53 MEDLINE on STN

2003086574. PubMed ID: 12598373. Biologic therapies for juvenile arthritis. Wilkinson N; Jackson G; Gardner-Medwin J. (Department of Rheumatology, Great Ormond Street Hospital, London, UK.. petherton@clara.co.uk) . Archives of disease in childhood, (2003 Mar) 88 (3) 186-91. Ref: 75. Journal code: 0372434. ISSN: 1468-2044. Pub. country: England: United Kingdom. Language: English.

AB A group of therapies with exciting potential has emerged for children and young people with severe juvenile idiopathic arthritis (JIA) uncontrolled by conventional disease modifying drugs. Theoretical understanding from molecular biologic research has identified specific targets within pathophysiological pathways that control rheumatoid arthritis (RA) and

JIA. This review identifies the pathways of autoimmunity to begin to show how biologic agents have been produced to replicate, mimic, or block culpable molecules and so promote or inhibit **cellular** activity or proliferation. Of these agents, cytokine antagonists have shown greatest promise, and early clinical studies of tumour necrosis factor (TNF) blockade have identified dramatic clinical benefit in many children with JIA. However, as will also be discussed, overlap of pathways within a complex immune system makes clinical response unpredictable and raises additional ethical and administrative concerns.

L28 ANSWER 10 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:486120 The Genuine Article (R) Number: 684UB. Quantitative interplay between activating and pro-apoptotic signals dictates T cell responses. Chen A S; Zheng G X; Tykocinski M L (Reprint). Univ Penn, Dept Pathol & Lab Med, 6 Gates Bldg, 3400 Spruce St, Philadelphia, PA 19104 USA (Reprint); Univ Penn, Dept Pathol & Lab Med, Philadelphia, PA 19104 USA. CELLULAR IMMUNOLOGY (FEB 2003) Vol. 221, No. 2, pp. 128-137. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0008-8749. Pub. country: USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Antigen-presenting cells (APC) can express surface ligands with both T cell activating and inhibitory capacities, prompting the question of how responding T cells integrate opposing trans signals concurrently delivered by APC. To address this question in a quantitative fashion, we turned to protein transfer as a unique experimental approach that is well-suited for addressing such questions from a quantitative standpoint. Costimulatory (either B7-1 . Fc(gamma1) or Fc(gamma1) . 4-1 BBL) and pro-apoptotic (Fc(gamma1) . FasL) **Fc fusion proteins** were quantitatively "painted" in varying ratios onto surrogate APC pre-coated with palmitated-protein A, the latter serving as a surface anchor. Evaluating the signaling potential of these various painted cells in a standard in vitro T cell proliferation assay, we demonstrated that at a given level of TCR triggering, the quantitative balance between costimulator (B7-1 or 4-1BBL) and FasL dictates the magnitude of the proliferative T cell response. Furthermore, when the costimulator density is kept constant, there is also a quantitative balance between TCR-directed and FasL signals. Interesting species-specific naive versus memory T cell subset differences emerged with regard to susceptibility to Fas-mediated apoptosis and costimulator:FasL opposition. Taken together, these data demonstrate for the first time a quantitative interplay between activating and pro-apoptotic tracts signals that dictates the magnitude of T cell responses.

L28 ANSWER 11 OF 53 MEDLINE on STN DUPLICATE 3
2003047102. PubMed ID: 12556207. BAFF: B cell survival factor and emerging therapeutic target for autoimmune disorders. Kalled Susan L; Ambrose Christine; Hsu Yen-Ming. (Department of Immunology and Inflammation, Biogen, Inc., 12 Cambridge Center, Cambridge, MA 02142, USA.. susan_kalled@biogen.com) . Expert opinion on therapeutic targets, (2003 Feb) 7 (1) 115-23. Journal code: 101127833. ISSN: 1472-8222. Pub. country: England: United Kingdom. Language: English.

AB The prevailing treatment strategies for autoimmune disorders employ global immunosuppressants that have harmful side effects with long-term use. A new vision for drug development relies on the generation of therapeutics that have specific and narrow targets, such as pathogenic cell populations. The **cellular** processes that initiate and maintain B cell dysregulation are not well understood and autoimmune disease results, in part, from the survival and activation of self-reactive B cells. Such B cells produce tissue-damaging pathogenic autoantibodies. BAFF (B cell-activating factor belonging to the TNF family), a member of the TNF family of ligands, may play a role in B cell-mediated diseases. BAFF is a survival factor for peripheral B cells. When BAFF is overexpressed in mice, B cell number and immunoglobulin production is

increased and an autoimmune-like phenotype is observed. Mouse models of lupus-nephritis have been shown to exhibit increased serum BAFF levels correlating with disease severity, and many autoimmune patients were found to have higher levels of circulating BAFF than healthy volunteers. Thus, modulating the level and activity of BAFF in these patients may alleviate symptoms associated with their disease. Several potential therapeutic inhibitors targeting BAFF are under investigation, including an anti-BAFF antibody and **receptor-Fc fusion proteins.**

L28 ANSWER 12 OF 53 MEDLINE on STN

2003426614. PubMed ID: 12966659. [From basic research to clinical results. The OVERTURE, ENABLE, and RENEWAL studies]. De la investigacion basica a los resultados clinicos. Estudios OVERTURE, ENABLE y RENEWAL. Pastelin Hernandez Gustavo; del Valle Mondragon Leonardo; Tenorio Lopez Fermin Alejandro. (Departamento de Formacologia, Instituto Nacional de Cardiologia Ignacio Chavez, INCICH, Juan Badiano No. 1, Col. Seccion XVI, Tlalpan, 14080 Mexico, D.F.. Gpastelin@aol.com) . Archivos de cardiologia de Mexico, (2003 Apr-Jun) 73 Suppl 1 S112-5. Journal code: 101126728. ISSN: 1405-9940. Pub. country: United States. Language: Spanish.

AB The results of three clinical studies (OVERTURE, ENABLE and RENEWAL), in patients with cardiac failure, are analyzed from a pharmacological point of view. In the first one of these, the action of an Angiotensin Converting Enzyme inhibitor, that at the same time inhibits the neutral endopeptidase, is studied. In the second, a blockade for endothelin **cellular receptors** is studied and, in the third, a synthetic acceptor of the alpha-Tumoral Necrosis Factor is taken into account. In the OVERTURE study, the benefit action of the inhibition of the Angiotensin Converting Enzyme in patients suffering from cardiac failure is confirmed, without a major effect from the neutral endopeptidase derived from its simultaneous inhibition. The other two studies were suspended because of the major side effects. The drugs used in OVERTURE, ENABLE and RENEWAL studies are relevant efforts of molecular design that, without any question, will project into the future of the therapeutic approach of cardiac failure. It is convenient to point out that in the task of designing clinical studies considering **cellular** signaling systems, there are other venues warranting their use in pathological or natural functions.

L28 ANSWER 13 OF 53 MEDLINE on STN

2003040994. PubMed ID: 12544029. Blockage of immune-mediated inner ear damage by etanercept. Wang Xiaobo; Truong Tim; Billings Peter B; Harris Jeffrey P; Keithley Elizabeth M. (Division of Otolaryngology-Head and Neck Surgery, University of California and Research Service of the Department of Veterans Affairs, San Diego, 92093-0666, USA.) Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology, (2003 Jan) 24 (1) 52-7. Journal code: 100961504. ISSN: 1531-7129. Pub. country: United States. Language: English.

AB HYPOTHESIS: Etanercept will be able to reduce the inflammation and hearing loss associated with experimentally induced labyrinthitis. BACKGROUND: Inner ear immune responses cause hearing loss that may be reversible with pharmacologic treatment. Etanercept, tumor necrosis factor **receptor** blocker, was investigated in a guinea pig model of immune-mediated hearing loss. Sterile labyrinthitis was created by injection of keyhole limpet hemocyanin into the inner ear after systemic sensitization to keyhole limpet hemocyanin with adjuvant. Labyrinthitis involves infiltration of inflammatory cells and hearing loss detectable 3 to 5 days after challenge with keyhole limpet hemocyanin. METHODS: Etanercept was administered either systemically (2.5 mg) 30 minutes before intracochlear challenge with keyhole limpet hemocyanin, with a second intraperitoneal dose (2.5 mg) 3 days later or locally by long-term infusion into the scala tympani with an osmotic pump (5.0 microg/h for 7

days). Auditory evoked brainstem response thresholds were measured before and after treatment to determine hearing loss. Cochleas were evaluated for the amount of inflammation. RESULTS: Hearing loss in the untreated systemic group averaged 71 +/- 21 dB versus 37 +/- 32 dB in the etanercept-treated animals (t test, $P < 0.001$). There was also less inflammation in the cochleas from etanercept-treated animals (t test, $P < 0.01$). Hearing loss with local administration of etanercept was 59 +/- 31 dB in the nontreated ears and 18 +/- 8 dB in the treated ears (t test, $P < 0.02$). Inflammation was also less (t test, $P < 0.01$). Etanercept was not ototoxic. CONCLUSION: Prompt intervention with the anti-inflammatory drug etanercept significantly reduces inflammation sufficient for substantive hearing preservation.

L28 ANSWER 14 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN
2002:429403 Document No. 137:2752 Monoclonal antibodies for the detection of decoy **receptor** 3-assocd. disease, hybridomas producing said antibodies and DcR3-IgG1 **Fc fusion proteins** for therapy. Mai, Shen-Chih; Liu, Shih-Jen (Taiwan). U.S. Pat. Appl. Publ. US 2002068064 A1 20020606, 12 pp. (English). CODEN: USXXCO. APPLICATION: US 2001-998196 20011203. PRIORITY: TW 2000-89125856 20001205.

AB The invention provides monoclonal antibodies against decoy **receptor** 3 (DcR3), hybridomas producing said antibodies, kits containing said monoclonal antibodies and uses of the hybridomas, antibodies and kits for the detection of DcR3-associated diseases, as well as for the treatment and/or prevention of DcR3-associated diseases. The use of fusion proteins consisting of DcR3 and IgG1 Fc fragment for treatment and prevention of DcR3-associated diseases is also discussed.

L28 ANSWER 15 OF 53 MEDLINE on STN DUPLICATE 4
2002372156. PubMed ID: 12117812. Ephrin-B ligands play a dual role in the control of neural crest cell migration. Santiago Alicia; Erickson Carol A. (Section of Molecular and Cellular Biology, University of California-Davis, Davis, California 95616, USA.. asantiagfo@ucdavis.edu) . Development (Cambridge, England), (2002 Aug) 129 (15) 3621-32. Journal code: 8701744. ISSN: 0950-1991. Pub. country: England: United Kingdom. Language: English.

AB Little is known about the mechanisms that direct neural crest cells to the appropriate migratory pathways. Our aim was to determine how neural crest cells that are specified as neurons and glial cells only migrate ventrally and are prevented from migrating dorsolaterally into the skin, whereas neural crest cells specified as melanoblasts are directed into the dorsolateral pathway. Eph **receptors** and their ephrin ligands have been shown to be essential for migration of many cell types during embryonic development. Consequently, we asked if ephrin-B proteins participate in the guidance of melanoblasts along the dorsolateral pathway, and prevent early migratory neural crest cells from invading the dorsolateral pathway. Using **Fc fusion proteins**, we detected the expression of ephrin-B ligands in the dorsolateral pathway at the stage when neural crest cells are migrating ventrally. Furthermore, we show that ephrins block dorsolateral migration of early-migrating neural crest cells because when we disrupt the Eph-ephrin interactions by addition of soluble ephrin-B ligand to trunk explants, early neural crest cells migrate inappropriately into the dorsolateral pathway. Surprisingly, we discovered the ephrin-B ligands continue to be expressed along the dorsolateral pathway during melanoblast migration. RT-PCR analysis, in situ hybridisation, and cell surface-labelling of neural crest cell cultures demonstrate that melanoblasts express several EphB **receptors**. In adhesion assays, engagement of ephrin-B ligands to EphB **receptors** increases melanoblast attachment to fibronectin. Cell migration assays demonstrate that ephrin-B ligands stimulate the migration of melanoblasts. Furthermore, when Eph signalling is disrupted in vivo, melanoblasts are

prevented from migrating dorsolaterally, suggesting ephrin-B ligands promote the dorsolateral migration of melanoblasts. Thus, transmembrane ephrins act as bifunctional guidance cues: they first repel early migratory neural crest cells from the dorsolateral path, and then later stimulate the migration of melanoblasts into this pathway. The mechanisms by which ephrins regulate repulsion or attraction in neural crest cells are unknown. One possibility is that the **cellular** response involves signalling to the actin cytoskeleton, potentially involving the activation of Cdc42/Rac family of GTPases. In support of this hypothesis, we show that adhesion of early migratory cells to an ephrin-B-derivatized substratum results in cell rounding and disruption of the actin cytoskeleton, whereas plating of melanoblasts on an ephrin-B substratum induces the formation of microspikes filled with F-actin.

L28 ANSWER 16 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2002:946350 The Genuine Article (R) Number: 617EC. Cytokines and their antagonists as therapeutic agents. Stevceva L (Reprint). Thomas Jefferson Med Coll, Dept Pathol Anat & Cell Biol, Jefferson Allumni Hall, Room 219, 1020 Locust St, Philadelphia, PA 19107 USA (Reprint); Thomas Jefferson Med Coll, Dept Pathol Anat & Cell Biol, Philadelphia, PA 19107 USA. CURRENT MEDICINAL CHEMISTRY (DEC 2002) Vol. 9, No. 24, pp. 2201-2207. Publisher: BENTHAM SCIENCE PUBL LTD. PO BOX 1673, 1200 BR HILVERSUM, NETHERLANDS. ISSN: 0929-8673. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cytokines are powerful molecules that the body's immune cells secrete in response to an offending agent. Their main function is to direct the immune response into the most effective pathway that will eventually result in elimination of the offender. The, last decade was marked by an enormous and ever growing interest that led to discovery of numerous cytokine molecules and their amazing influence on the body immune function. The more we are learning about the way cytokines modulate and direct the immune responses of the body, the interest in using them or their antagonist to change or enhance those responses is growing.

Studies are currently underway showing the beneficial effect of TNFalpha antagonists on the **cellular** injury mediated by this cytokine in rheumatic diseases, inflammatory bowel disease and endotoxemia. Interferon therapies are also tested utilizing IFNalpha for treatment of Hepatitis B and C. The discovery of Th1 and Th2 cytokines had shown that the nature of the immune response is, in essence, directed by a few important cytokines. Which immune reactions will develop seems to depend on whether IL-2 and IL-12 are secreted (and the immune response becomes Th1 with secretion of IFNgamma and efficient removal of some antigens such as viruses) or IL-4 is secreted in which case Th2 response results in down regulation of IFNgamma and IL-2 secreting effectors.

The discovery, isolation and purification of these molecules open the possibility to skew the immune response in order to facilitate better outcome. For example, studies have now being conducted aimed at using IL-2 as an adjuvant therapy in conjunction with HAART in HIV patients. Similarly, IL-12 seems to be beneficial in melanoma and has been used as a very potent adjuvant for eliciting immune responses to immunization. Furthermore, studies with IL-4 knockout mice and those utilizing IL-4 blocking agents have shown that this cytokine might play a crucial role in maintaining persistent viral infections and in mediating chronic, autoimmune diseases.

Using body's own immunomodulators is becoming an exciting possibility to target inefficient or misdirected immune responses that result in disease. The potential benefits in terms of human disease are enormous and still largely unexplained. Thus, using cytokines and their antagonists as therapeutic agents is an emerging and growing area of research.

L28 ANSWER 17 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2003096155 EMBASE BAFF: A novel therapeutic target for autoimmunity. Kalled

S.L.. S.L. Kalled, Biogen Inc., 12 Cambridge Center, Cambridge, MA 02142, United States. Susan_Kalled@biogen.com. Current Opinion in Investigational Drugs 3/7 (1005-1010) 1 Jul 2002.
Refs: 42.
ISSN: 1472-4472. CODEN: CIDREE. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Autoimmunity results from a break in self-tolerance involving humoral and/or cell-mediated immune mechanisms. One pathological consequence of a failure in central and/or peripheral tolerance is the generation of autoantibodies and subsequent formation of complement-fixing immune complexes that contribute to tissue damage. Prevailing pharmacological strategies for treating autoimmune diseases involve the use of broad-acting immunosuppressants that with long term use have associated toxicities. The current drive in drug development is towards therapies that target a specific biological pathway or pathogenic cell population. Recent discovery of the BAFF-mediated B-cell survival pathway provides a unique opportunity for developing focused intervention for autoreactive B-cell function.

L28 ANSWER 18 OF 53 MEDLINE on STN
2003058610. PubMed ID: 12568977. Elevation of soluble tumor necrosis factor **receptor** II in non-febrile patients with acute myeloid leukemia. Goetz M; Schiel X; Heussel H; Steinmetz T; Hiddemann W; Weiss M. (Medizinische Klinik, Universitat Mainz, Munchen, Germany.. mgoetz@mail.uni-mainz.de) . European journal of medical research, (2002 Nov 25) 7 (11) 487-90. Journal code: 9517857. ISSN: 0949-2321. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB BACKGROUND: Tumor necrosis factor alpha (TNF) and its **cellular** and soluble (s) **receptors** (TNF-R) are important mediators in acute myeloid leukemia (AML) and infectious complications during cytoreductive therapy. We investigated the serum concentrations of sTNF-RII in previously untreated patients with AML at the onset of cytoreductive therapy and in non-febrile chemotherapy-associated neutropenia. PATIENTS AND METHODS: Of 54 eligible patients with AML, serum concentrations of sTNF-RII could be evaluated in 25 non-neutropenic, non-febrile and in 11 neutropenic, non-febrile patients. RESULTS: At baseline, non-neutropenic, non-febrile AML patients showed high median serum sTNF-RII concentrations of 3,804 pg/mL. In neutropenia, there was a non-significant trend ($p = 0.18$) to lower median sTNF-RII levels of 3,246 pg/mL. CONCLUSIONS: Serum sTNF-RII concentrations in non-febrile AML patients before chemotherapy are in the range of levels reached in uncomplicated febrile episodes in otherwise healthy individuals. This must be taken into account when evaluating the cytokine profile for sepsis in patients with therapy-associated neutropenia. Concentrations are still elevated in neutropenia, suggesting that a normal number of leukocytes is not necessarily required for the activation of the TNF ligand/TNF **receptor** system in AML.

L28 ANSWER 19 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN
2003:359009 Document No. 138:350238 Construction of eukaryotic expression vector for fusion gene TGF-βRII/Fc and its expression in CHO cells. Li, Shengqing; Li, Huanzhang; Yang, Qiaoxin; Wang, Jicun; Ni, Diantao; Qi, Haowen (Xijing Hospital, Fourth Military Medical University, Xian, Shanxi Province, 710033, Peop. Rep. China). Disi Junyi Daxue Xuebao, 23(4), 371-375 (Chinese) 2002. CODEN: DJDXEG. ISSN: 1000-2790. Publisher: Disi Junyi Daxue Xuebao Bianjibu.

AB The eukaryotic expression vector pIg-TRII for the fusion gene expressing bioactive extracellular domain of human transforming growth factor beta type II **receptor** (TGF-βR II) and Ig Fc fragment was constructed and the chimeric protein TGF-βR II/Fc was expressed in CHO cells. Total RNA was extracted from human normal lung tissue and subjected to reverse transcription, and then the human TGF-βRII cDNA gene was amplified by RT-PCR. The PCR products was cloned into pMD18-T

vector and the sequence was confirmed by restriction enzyme digestion and sequencing. Then the specific TGF-βRII coding region fragment was subcloned into pIg plasmid to form the pIg-TRII eukaryotic expression vector. The recombinant plasmid was transfected into CHO cells with liposome transfection reagent for transient expression. Restriction enzyme digestion and DNA sequence anal. revealed that the insert fragment was identical to the published TGF-βRII cDNA sequence. The chimeric protein was detected by **cellular** immunofluorescence and immunopptn. technol. Thus, we have successfully constructed the pIg-TRII eukaryotic expression vector for the fusion gene TGF-βRII/Fc and expressed it in CHO cells. This work would lay the foundation of gene therapy of pulmonary fibrosis.

L28 ANSWER 20 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN
2002:268259 Document No. 137:4864 Blockade of T cell Costimulatory Signals using Adenovirus Vectors Prevents both the Induction and the Progression of Experimental Autoimmune Myocarditis. Matsui, Yutaka; Inobe, Manabu; Okamoto, Hiroshi; Chiba, Satoru; Shimizu, Toshihiro; Kitabatake, Akira; Uede, Toshimitsu (Division of Molecular Immunology, Institute for Genetic Medicine, Hokkaido University, Sapporo, 060-0815, Japan). Journal of Molecular and Cellular Cardiology, 34(3), 279-295 (English) 2002. CODEN: JMCDAY. ISSN: 0022-2828. Publisher: Academic Press.
AB Exptl. autoimmune myocarditis (EAM) has been used as a model for human myocarditis in relation to the autoimmune mechanism and proved to be a T cell-mediated autoimmune disease. Interactions of T cell surface **receptors** CD28 and CD40L with their ligands B7 and CD40, resp., on APCs are critical for antigen-specific T cell activation under physiol. and pathol. conditions. To achieve effective inhibition of these interactions, we have constructed adenovirus vectors containing CTLA4Ig (AdexCTLA4Ig) and CD40Ig (AdexCD40Ig) and examined the effects of these adenovirus vectors in preventing EAM. AdexLacZ as a control, or AdexCTLA4Ig and/or AdexCD40Ig were injected i.v. into rats on day 0 or 14 after immunization to study the preventive effects on EAM in the T cell activation phase or inflammatory phase. Disease severity was estimated by the macroscopic and microscopic findings of the heart, heart weight to body weight ratios, and **cellular** and humoral immune responses on day 21. The onset of EAM after AdexCTLA4Ig or AdexCD40Ig treatment on day 0 was completely inhibited and antigen-specific lymphocyte proliferation was significantly reduced in those adenovirus-treatment groups, suggesting that those therapies induce antigen-specific T cell anergy. Moreover, significant reduction in disease severity was achieved after the adenovirus vector treatment even on day 14 compared with EAM rats. This study indicates the therapeutic potential of costimulatory pathway blockade by gene-transfer in myocarditis. (c) 2002 Academic Press.

L28 ANSWER 21 OF 53 MEDLINE on STN
2002720503. PubMed ID: 12482385. Recombinantly engineered human proteins: transforming the treatment of psoriasis. Gottlieb Alice B; Bos Jan D. (Clinical Research Center, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick 08901, USA.. gottliab@umdnj.edu) . Clinical immunology (Orlando, Fla.), (2002 Nov) 105 (2) 105-16. Ref: 81. Journal code: 100883537. ISSN: 1521-6616. Pub. country: United States. Language: English.
AB Psoriasis is a chronic, inflammatory disease with lesions that produce considerable physical discomfort and often lead to substantial disruption in patients' daily activities. The use of currently available, nonspecific, systemic immunosuppressive therapies for patients with moderate to severe psoriasis is limited by an inability to maintain disease remission safely. Advances in recombinant DNA technology paralleled with increased understanding of the immunopathology of psoriasis have led to the development of numerous biologic agents for the treatment of this disease. These new biologic therapies target specific steps in psoriasis pathology, including direct effects on T cells, T cell

activation, T cell migration, and **cellular** production and secretion of cytokines. By selectively targeting the activities of T cells that are directly involved in psoriasis pathogenesis, these novel agents offer improved safety profiles and enhanced efficacy. In this article, the mechanisms of T cell pathogenicity that guided the development of these new biologic therapies are reviewed along with clinical data on the progress of these agents.

L28 ANSWER 22 OF 53 EMBASE
on STN
2001425974 EMBASE Detection and localization of cripto-1 binding in mouse mammary epithelial cells and in the mouse mammary gland using an immunoglobulin - Cripto-1 fusion protein. Bianco C.; Normanno N.; De Luca A.; Maiello M.R.; Wechselberger C.; Sun Y.; Khan N.; Adkins H.; Sanicola M.; Vonderhaar B.; Cohen B.; Seno M.; Salomon D.. D. Salomon, TGFS, BRL, NCI, 9000 Rockville Pike, Bethesda, MD 20892, United States. davetgfa@helix.nih.gov. Journal of Cellular Physiology 190/1 (74-82) 2002.
Refs: 41.
ISSN: 0021-9541. CODEN: JCLLAX. Pub. Country: United States. Language: English. Summary Language: English.
AB Human Cripto-1 (CR-1), a member of the epidermal growth factor-CFC (EGF-CFC) family of peptides, is expressed in the developing mouse mammary gland and can modulate mammary epithelial cell migration, branching morphogenesis and milk protein expression in vitro. In order to screen for a CR-1 **receptor** and to identify potential CR-1 target tissues, we constructed a fusion protein comprising the EGF-like domain of CR-1 and the Fc domain of a human IgG1. The recombinant CR-1 fusion protein (CR-1-Fc) was biologically active as it was able to activate the ras/raf/mitogen activated protein kinase (MAPK) pathway and to inhibit transcription of the milk protein β-casein in NMuMG and HC-11 mouse mammary epithelial cells. By using immunocytochemistry and by an in situ enzyme-linked immunosorbent assay (ELISA), CR-1-Fc was found to specifically bind to NMuMG and HC-11 cells. Finally, immunohistochemical analysis using CR-1-Fc showed a specific localization of CR-1 binding to tissue sections from mouse mammary gland. In particular, more than 60% of the epithelial cells were intensely stained with the CR-1-**Fc fusion protein** in the lactating mouse mammary gland, whereas approximately 25% of the mammary epithelial cells were stained in the gland from pregnant mouse. Since expression of mouse cripto-1 (Ct-1) in the pregnant and lactating mouse mammary gland as well as its presence in milk has been previously demonstrated, these data strongly suggest that an autocrine pathway involving Cr-1 and its putative **receptor** is operating in the mouse mammary gland during pregnancy and lactation.

L28 ANSWER 23 OF 53 MEDLINE on STN
2002474691. PubMed ID: 12236617. The mode of action of cytokine inhibitors. Arend William P. (University of Colorado Health Sciences Center, Denver 80262, USA.. william.arend@uchsc.edu) . Journal of rheumatology. Supplement, (2002 Sep) 65 16-21. Ref: 43. Journal code: 7806058. ISSN: 0380-0903. Pub. country: Canada. Language: English.
AB Tumor necrosis factor-alpha (TNF-alpha) and interleukin 1 (IL-1) are important mediators of inflammation and tissue damage in animal models of inflammatory arthritis and in patients with active rheumatoid arthritis (RA). Several inhibitors of these cytokines are now available for RA treatment, each having a different mode of action. Etanercept is a recombinant fusion protein of the soluble type II TNF **receptor** on a human IgG1 backbone, whereas infliximab is a chimeric anti-TNF-alpha monoclonal antibody containing a murine TNF-alpha binding region and human IgG1 backbone. Both agents potently and selectively bind TNF-alpha in the **cellular** microenvironment, thereby preventing TNF-alpha from interacting with membrane-bound TNF **receptors** on target cells. In comparison, anakinra is a recombinant human IL-1 **receptor**

antagonist (IL-1Ra) that binds avidly to type 1 IL-1 **receptors** but does not stimulate any intracellular responses. Studies of these agents in animal models of inflammatory arthritis suggest that TNF-alpha plays a more important role in promoting inflammation, whereas IL-1 is more important in causing cartilage and bone destruction. However, these differential actions have not been borne out in clinical trials, where TNF-alpha blockers and anakinra similarly reduce clinical signs and symptoms of RA as well as slow radiographic evidence of disease progression.

L28 ANSWER 24 OF 53 MEDLINE on STN DUPLICATE 5

2001271977. PubMed ID: 11325842. Targeting dendritic cells to enhance DNA vaccine potency. You Z; Huang X; Hester J; Toh H C; Chen S Y. (Center for Cell and Gene Therapy, Departments of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA.) Cancer research, (2001 May 1) 61 (9) 3704-11. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB DNA vaccination that can induce both **cellular** and humoral immune responses has become an attractive immunization strategy against cancer and infection. Dendritic cells (DCs) play a critical role in the induction of immune responses by DNA vaccination. However, a major problem of DNA vaccination is its limited potency, because only a very limited fraction of injected DNA molecules are taken up by DCS: In this study, we describe a novel DNA vaccination strategy to enhance uptake and presentation of antigens by DCS: Specifically, we developed a DNA vaccine based upon expression of a model hepatitis B virus (HBV) e antigen fused to an IgG Fc fragment. After vaccination, the DNA are taken up by cells that produce and secrete the antigen-**Fc fusion proteins**. The secreted fusion proteins, in addition to inducing B cells, are efficiently captured and processed by DCs via **receptor** -mediated endocytosis and then presented to the MHC class II and as -I (cross-priming). The results of this study demonstrate that broad enhancement of antigen-specific CD4+ helper, CD8+ cytotoxic T-cell, and B-cell responses can be achieved by this DNA vaccination strategy. Thus, the strategy capable of inducing all arms of the adaptive immunity may provide a novel, generic design for the development of therapeutic and preventive DNA vaccines.

L28 ANSWER 25 OF 53 MEDLINE on STN DUPLICATE 6

2001395137. PubMed ID: 11294894. Layilin, a novel integral membrane protein, is a hyaluronan **receptor**. Bono P; Rubin K; Higgins J M; Hynes R O. (Howard Hughes Medical Institute, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139, USA.) Molecular biology of the cell, (2001 Apr) 12 (4) 891-900. Journal code: 9201390. ISSN: 1059-1524. Pub. country: United States. Language: English.

AB The actin cytoskeleton plays a significant role in changes of cell shape and motility, and interactions between the actin filaments and the cell membrane are crucial for a variety of **cellular** processes. Several adaptor proteins, including talin, maintain the cytoskeleton-membrane linkage by binding to integral membrane proteins and to the cytoskeleton. Layilin, a recently characterized transmembrane protein with homology to C-type lectins, is a membrane-binding site for talin in peripheral ruffles of spreading cells. To facilitate studies of layilin's function, we have generated a layilin-**Fc fusion protein** comprising the extracellular part of layilin joined to human immunoglobulin G heavy chain and used this chimera to identify layilin ligands. Here, we demonstrate that layilin-**Fc fusion protein** binds to hyaluronan immobilized to Sepharose. Microtiter plate-binding assays, coprecipitation experiments, and staining of sections predigested with different glycosaminoglycan-degrading enzymes and cell adhesion assays all revealed that layilin binds specifically to hyaluronan but not to other tested glycosaminoglycans. Layilin's ability to bind hyaluronan, a ubiquitous extracellular matrix

component, reveals an interesting parallel between layilin and CD44, because both can bind to cytoskeleton-membrane linker proteins through their cytoplasmic domains and to hyaluronan through their extracellular domains. This parallelism suggests a role for layilin in cell adhesion and motility.

L28 ANSWER 26 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN
2002:120740 Document No. 136:261621 Gamma-interferon induces apoptosis of the B lymphoma WEHI-279 cell line through a CD95/CD95L-independent mechanism. Ben Jilani, K.-E.; Akarid, K.; Arnoult, D.; Petit, F.; Baert, E.; Gaillard, J.-P.; Ameisen, J.-C.; Estaquier, J. (Department of Adult Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, 02115, USA). European Cytokine Network, 12(4), 587-596 (English) 2001. CODEN: ECYNEJ. ISSN: 1148-5493. Publisher: John Libbey Eurotext.
AB Gamma-interferon (IFN-γ) a cytokine produced by CD4+ T helper type 1 cells, CD8+ T cells and natural killer (NK) cells, plays a central role in the development of humoral and cell-mediated immunity. IFN-γ participates in the maturation and differentiation of B cells, but it has been previously reported that IFN-γ may inhibit the early stages of B cell activation. We report that the inhibition of the B lymphoma cell WEHI-279-proliferation induced by IFN-γ, involves the induction of typical features of apoptosis (nuclear chromatin condensation and fragmentation, cell shrinkage, phosphatidyl-serine (PS) exposure and mitochondrial membrane potential (Δψm) loss).
IFN-γ-mediated B cell apoptosis was decreased by the addition of the T helper type 2 cytokine, IL-4. WEHI-279 cells express CD95 and undergo apoptosis after treatment with either an agonistic anti-CD95 Ab or with a soluble recombinant CD95L. However, incubation with CD95-Fc or TRAIL-R1-**Fc fusion proteins**, did not prevent
IFN-γ-mediated apoptosis, suggesting that IFN-γ-mediated apoptosis occurs independently of CD95/CD95L and TRAIL-R/TRAIL interactions. IFN-γ-mediated apoptosis is associated with caspase-3 activation that can be prevented by the addition of the broad caspase inhibitor zVAD-fmk. These data indicate that IFN-γ may play a major role in the regulation of B cell apoptosis, and suggest the involvement of an alternative pathway which is independent of the death **receptors**
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L28 ANSWER 27 OF 53 MEDLINE on STN
2001380392. PubMed ID: 11436681. [The role of cell-mediated immune response in pathogenesis of alopecia areata]. Rola odpowiedzi immunologicznej typu komorkowego w patogenezie lysienia plackowatego. Lis A; Pierzchala E; Brzezinska-Wcislo L. (Katedry i Kliniki Dermatologii Slaskiej Akademii Medycznej w Katowicach.) Wiadomosci lekarskie (Warsaw, Poland : 1960), (2001) 54 (3-4) 159-63. Journal code: 9705467. ISSN: 0043-5147. Pub. country: Poland. Language: Polish.
AB Recent studies have suggested that cell-mediated immune response play a critical role in the pathogenesis of alopecia areata (AA). Eighteen patients with AA were included in the study. Fifteen healthy subjects served as controls. Serum levels of sTNF alpha RI and sIL-2R were measured using enzyme-linked immunosorbent assay technique. The serum levels of sTNF alpha RI were significantly elevated in patients with AA in comparison with control group. The serum levels of sIL-2R were higher in AA patients than in healthy subjects but not significantly. These results indicate, that immune mechanisms in AA are characterized by activation of T cells and other cells, possibly keratinocytes.

L28 ANSWER 28 OF 53 MEDLINE on STN DUPLICATE 7
2001494588. PubMed ID: 11326474. Tumor necrosis factor-alpha and **receptors** for it in labial salivary glands in Sjogren's syndrome. Koski H; Janin A; Humphreys-Beher M G; Sorsa T; Malmstrom M; Konttinen Y T. (Institute of Biomedicine, Department of Anatomy, University of Helsinki, Finland.. hannele.koski@helsinki.fi) . Clinical and experimental

rheumatology, (2001 Mar-Apr) 19 (2) 131-7. Journal code: 8308521. ISSN: 0392-856X. Pub. country: Italy. Language: English.

AB OBJECTIVE: Modulation of TNF-alpha by neutralizing antibodies, soluble **receptors** and TNFR: **Fc fusion proteins** are being developed for the therapeutic modulation of immune inflammation. It is becoming increasingly important to understand the state and involvement of the TNF-alpha/TNFR system in various rheumatic diseases. Tumor necrosis factor-alpha (TNF-alpha) affects its target cells through binding to two different **receptors**, TNFR-p55 and TNFR-p75. Mitogenic, cytostatic and cytotoxic effects of TNF-alpha on various cells have been reported. In Sjogren's syndrome (SS) focal sialadenitis leads to salivary gland destruction and loss of function. Although TNF-alpha is one possible mediator in these processes, nothing is known about the spatial distribution of TNF-alpha in relation to its **receptors** /target cells in salivary gland tissue. METHODS: Labial salivary glands (LSG) were obtained from 16 SS patients and 13 healthy controls and stained using the immunohistochemical peroxidase-anti-peroxidase (PAP) method for TNF-alpha, TNFR-p55 and TNFR-p75. RESULTS: TNF-alpha, TNFR-p55 and TNFR-p75 staining was absent, weak or relatively inextensive in controls compared to SS patients. Infiltrating mononuclear inflammatory cells in SS patients displayed moderate to strong TNF-alpha and TNFR expression. In addition, resident vascular endothelial cells, ductal epithelial cells and fibroblasts co-expressed TNF-alpha and TNFR. In contrast, acinar end piece cells did not express TNF-alpha or TNFR-p75 although TNFR-p55 was expressed. CONCLUSION: The interrelated localization of TNF **receptors** and their ligand TNF-alpha in inflammatory and in endothelial cells suggests a proinflammatory role of TNF-alpha in SS. The expression of TNF-alpha and its **receptors** in fibroblasts and ductal cells may contribute to ductal hyperplasia and glandular fibrosis. However, in contrast to expectations, the **cellular** localization of the TNF-alpha/TNRF system argues against its role in acinar cell atrophy.

L28 ANSWER 29 OF 53 EMBASE on STN

2001198192 EMBASE Inactivation of multiple targets by nitric oxide in CD95-triggered apoptosis. Bernassola F.; Catani M.V.; Corazzari M.; Rossi A.; Melino G.. G. Melino, IDI-IRCCS, Biochemistry Lab, University of Rome Tor Vergata, Via Tor Vergata 135, 00133 Rome, Italy. gerry.melino@uniroma2.it. Journal of Cellular Biochemistry 82/1 (123-133) 2001.
Refs: 55.
ISSN: 0730-2312. CODEN: JCEBD5. Pub. Country: United States. Language: English. Summary Language: English.

AB Nitric oxide (NO) plays an important anti-apoptotic role by inactivating both upstream and downstream apoptotic molecules. We now report that exogenously supplied NO protected Jurkat T cells from anti-CD95-stimulated apoptosis. We have recently shown that nitrosation of the activator protein-1 (AP-1) transcriptional factor is crucial for NO-mediated inhibition of cell death triggered by etoposide or ceramide. Since the inhibition of apoptosis by NO has been reported to involve AP-1, we evaluated its involvement in in CD95-mediated cell death. Cross-linking of CD95 enhanced AP-1 DNA binding activity and AP-1-dependent CD95L transactivation, which were both significantly reduced by different NO-donors compounds. However, AP-1 induction does not seem to significantly contribute to anti-CD95-triggered apoptosis, as cell death could not be prevented by using the recombinant Fas-**Fc** **fusion protein** which inhibits the CD95/CD95L interaction. We observed that caspase 3-like activity was negatively modulated by several NO-donors in vitro and that titratable thiol groups of purified caspases 3, 7, and 9 decreased in the presence of NO-releasing compounds. In conclusion, we demonstrated that NO-mediated inhibition of other targets, possibly caspases, but not AP-1, is a crucial event

responsible for protection against anti-CD95-stimulated apoptosis. Even though NO affects multiple molecular mechanisms, the relevant target for exerting the **cellular** effects, may vary among different models. .COPYRGT. 2001 Wiley-Liss, Inc.

L28 ANSWER 30 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN
2001:242658 Document No. 135:91168 Expression of single-chain Fv-Fc fusions in Pichia pastoris. Powers, D. B.; Amersdorfer, P.; Poul, M.-A.; Nielsen, U. B.; Shalaby, M. R.; Adams, G. P.; Weiner, L. M.; Marks, J. D. (Departments of Anesthesia and Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, 94110, USA). Journal of Immunological Methods, 251(1-2), 123-135 (English) 2001. CODEN: JIMMBG. ISSN: 0022-1759. Publisher: Elsevier Science B.V..

AB Phage display technol. makes possible the direct isolation of monovalent single-chain Fv antibody fragments. For many applications, however, it is useful to restore Fc mediated antibody functions such as avidity, effector functions and a prolonged serum half-life. The authors have constructed vectors for the convenient, rapid expression of a single-chain antibody Fv domain (scFv) fused to the Fc portion of human IgG1 in the methylotrophic yeast Pichia pastoris. The scFv-**Fc fusion protein** is secreted and recovered from the culture medium as a disulfide-linked, glycosylated homodimer. The increased size of the dimer (.apprx.106 kDa vs. .apprx.25 kDa for a scFv) results in a prolonged serum half-life in vivo, with t1/2 of the beta phase of clearance increasing from 3.5 h for a typical scFv to 93 h for a scFv-Fc fusion in mice. The scFv-Fc fusion is capable of mediating antibody-dependent **cellular** cytotoxicity against tumor target cells using human peripheral blood mononuclear cells as effectors. Finally, the Fc domain is a convenient, robust affinity handle for purification and immunochem. applications, eliminating the need for proteolytically sensitive epitope and/or affinity tags on the scFv.

L28 ANSWER 31 OF 53 MEDLINE on STN DUPLICATE 8
2002017559. PubMed ID: 11436637. Cloning and expression of human TNFR (P55)-IgG **Fc fusion protein** in eukaryotic cells. An N; Xu C; Zhang Z. (State Key Laboratory of Molecular Virology and Genetic Engineering, Institute of Virology, Chinese Academy of Preventive Medicine, Beijing 100052, China.) Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology, (2001 Jun) 15 (2) 116-7. Journal code: 9602873. ISSN: 1003-9279. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To express soluble biologically active TNF **receptor** protein in eukaryotic cells, which can inhibit cytotoxic activity of TNF. METHODS: PCR amplified the extra-**cellular** region of TNF **receptor** P55 and IgG Fc gene. Then the two were linked through an oligomer encoding a thrombin-sensitive peptide linker and cloned into eukaryotic expression vector pcDNA3.1 (+). The eukaryotic expression plasmid pcDNA3. 1/TI was then transfected to the mammalian cell line COS7 and BHK. Using the G418 system, BHK cell clones were selected and can continuously secrete biological protein in large amount. RESULTS: The expressed protein was fused with IgG Fc and secreted into the cell culture supernatant. It has good antigenicity and binding ability to TNF. It can also inhibit the cytotoxic activity of TNF on L929 cell. CONCLUSION: The TNFR-IgG **Fc fusion protein** expressed in eukaryotic cells has biological activities of human TNF **receptor** P55.

L28 ANSWER 32 OF 53 MEDLINE on STN
2002157694. PubMed ID: 11890650. Cytokine blockers in psoriatic arthritis. Mease P J. (Seattle Rheumatology Associates and Division of Clinical Research, Swedish Hospital Medical Centre, and University of Washington, 98104, USA.. pmease@u.washington.edu) . Annals of the rheumatic diseases,

(2001 Nov) 60 Suppl 3 iii37-40. Journal code: 0372355. ISSN: 0003-4967. Pub. country: England: United Kingdom. Language: English.

AB The **cellular** events underlying the pathogenesis of psoriatic arthritis (PsA) and psoriasis have not yet been fully elucidated. Nevertheless, some clues to these conditions are beginning to emerge. In particular, a growing body of data supports the role of proinflammatory cytokines, such as tumour necrosis factor (TNF), in the pathophysiology of PsA and psoriasis. Raised levels of these cytokines are found in the joints of patients with PsA, as well as in psoriatic skin lesions. Physiotherapy, non-steroidal anti-inflammatory agents, corticosteroids, and disease modifying antirheumatic agents, such as methotrexate, are the most commonly used treatments for PsA. However, the data supporting the effectiveness of these treatments are limited, and disease resolution is usually incomplete. This study examined the effects of etanercept, a TNF inhibitor, in patients with PsA. Etanercept treatment was well tolerated and resulted in significant improvement in the signs and symptoms of PsA and in psoriatic skin lesions. Infliximab, another TNF inhibitor, has also been shown to be effective in patients with PsA. Such studies confirm the importance of proinflammatory cytokines in PsA, and hold out hope for patients who require new options for the treatment of their disease.

L28 ANSWER 33 OF 53 MEDLINE on STN DUPLICATE 9

2001048384. PubMed ID: 10934210. Molecular and **cellular** properties of the rat AA4 antigen, a C-type lectin-like **receptor** with structural homology to thrombomodulin. Dean Y D; McGreal E P; Akatsu H; Gasque P. (Brain Inflammation and Immunity Group, Medical Biochemistry Department, University of Wales College of Medicine, Cardiff, CF144XN, United Kingdom.) Journal of biological chemistry, (2000 Nov 3) 275 (44) 34382-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The murine fetal stem cell marker AA4 has recently been cloned and is known to be the homolog of the human phagocytic C1q **receptor** involved in host defense. We herein report the molecular cloning and the **cellular** expression pattern of the rat AA4 antigen. Modular architecture analysis indicated that the rat AA4 is a member of C-type lectin-like family and, interestingly, displays similar domain composition and organization to thrombomodulin. Northern blot and reverse transcriptase-polymerase chain reaction analyses indicated that rat AA4 was encoded by a single transcript of 7 kilobases expressed constitutively in all tissues. In situ hybridization showed that AA4 was expressed predominantly by pneumocytes and vascular endothelial cells. Using an affinity purified polyclonal antibody raised against a rat AA4-**Fc fusion protein**, AA4 was identified as a glycosylated protein of 100 kDa expressed by endothelial cells > platelets > NK cells and monocytes (ED1+ cells). The staining was associated to the cell surface and intracytoplasmic vesicles. Conversely, erythrocytes, T and B lymphocytes, neutrophils, and macrophages (ED2+ cells) were consistently negative for AA4. As expected, the macrophage cell line NR8383 expressed weak levels of AA4. Taken together, our results support the idea that AA4/C1qRp is involved in some cell-cell interactions.

L28 ANSWER 34 OF 53 MEDLINE on STN DUPLICATE 10

2000487952. PubMed ID: 11035077. Reciprocal expression of the TNF family **receptor** herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own **receptor**. Morel Y; Schiano de Colella J M; Harrop J; Deen K C; Holmes S D; Wattam T A; Khandekar S S; Truneh A; Sweet R W; Gastaut J A; Olive D; Costello R T. (Laboratoire d'Immunologie des Tumeurs, Departement d'Hematologie, Institut Paoli Calmettes, Universite de la Mediterranee, Marseille, France.) Journal of immunology (Baltimore, Md. : 1950), (2000 Oct 15) 165 (8) 4397-404. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The TNF **receptor** (TNFR) family plays a central role in the development of the immune response. Here we describe the reciprocal regulation of the recently identified TNFR superfamily member herpes virus entry mediator (HVEM) (TR2) and its ligand LIGHT (TL4) on T cells following activation and the mechanism of this process. T cell activation resulted in down-regulation of HVEM and up-regulation of LIGHT, which were both more pronounced in CD8(+) than CD4(+) T lymphocytes. The analysis of HVEM and LIGHT mRNA showed an increase in the steady state level of both mRNAs following stimulation. LIGHT, which was present in cytoplasm of resting T cells, was induced both in cytoplasm and at the cell surface. For HVEM, activation resulted in **cellular** redistribution, with its disappearance from cell surface. HVEM down-regulation did not rely on de novo protein synthesis, in contrast to the partial dependence of LIGHT induction. Matrix metalloproteinase inhibitors did not modify HVEM expression, but did enhance LIGHT accumulation at the cell surface. However, HVEM down-regulation was partially blocked by a neutralizing mAb to LIGHT or an HVEM-**Fc fusion protein** during activation. As a model, we propose that following stimulation, membrane or secreted LIGHT binds to HVEM and induces **receptor** down-regulation. Degradation or release of LIGHT by matrix metalloproteinases then contributes to the return to baseline levels for both LIGHT and HVEM. These results reveal a self-regulating ligand/ **receptor** system that contributes to T cell activation through the interaction of T cells with each other and probably with other cells of the immune system.

L28 ANSWER 35 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000249973 EMBASE T1-deficient and T1-Fc-transgenic mice develop a normal protective Th2-type immune response following infection with Nippostrongylus brasiliensis. Senn K.A.; McCoy K.D.; Maloy K.J.; Stark G.; Frohli E.; Rulicke T.; Klemenz R.. R. Klemenz, Department of Pathology, Division of Cancer Research, University Hospital Zurich, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland. roman.klemenz@pty.usz.ch. European Journal of Immunology 30/7 (1929-1938) 2000.
Refs: 30.
ISSN: 0014-2980. CODEN: EJIMAF. Pub. Country: Germany. Language: English. Summary Language: English.

AB The IL-1 **receptor**-related protein T1 is expressed on the surface of Th2, but not Th1 cells. Studies with anti-T1 monoclonal antibodies have suggested that T1 is critical for development of normal Th2-type responses. To elucidate the role of T1 in vivo, we generated T1-deficient mice and a T1-transgenic strain which secretes soluble T1-**Fc fusion protein** into the serum. These were analyzed for the Th2 immune response induced by infection with the parasitic nematode Nippostrongylus brasiliensis. Although Th2 cytokine production by lymph node cells was similar in all groups of N. brasiliensis-infected mice, a decrease in IL-5 production by lung lymphocytes was detected in both T1-deficient and T1-Fc-transgenic mice compared to control littermates. This difference in IL-5 production did not influence blood eosinophilia, but recruitment of eosinophils into lung tissue, especially in T1-Fc-transgenic mice was slightly decreased. However, induction of all other immune parameters was normal and both T1-deficient and T1-Fc-transgenic mice were able to clear the parasite infection within 12 days with kinetics similar to those in control mice. Therefore, in contrast to previous suggestions, we conclude that the T1 protein is not obligatory for normal development of Th2 immune responses.

L28 ANSWER 36 OF 53 MEDLINE on STN DUPLICATE 11

2001043689. PubMed ID: 11027164. Effect of PDI overexpression on recombinant protein secretion in CHO cells. Davis R; Schooley K; Rasmussen B; Thomas J; Reddy P. (Departments of Cell Science and Biochemistry,

Immunex Corporation, 51 University Street, Seattle, Washington 98101, USA.) Biotechnology progress, (2000 Sep-Oct) 16 (5) 736-43. Journal code: 8506292. ISSN: 8756-7938. Pub. country: United States. Language: English.

AB In eukaryotic cells, protein disulfide isomerase (PDI) found in the endoplasmic reticulum (ER) catalyzes disulfide bond exchange and assists in protein folding of newly synthesized proteins. PDI also functions as a molecular chaperone and has been found associated with proteins in the ER. In addition, PDI functions as a subunit of two more complex enzyme systems: the prolyl-4-hydroxylase and the triacylglycerol transfer proteins. Increasing PDI activity in bacterial, yeast, and insect cell expression systems can lead to increased secretion of heterologous proteins containing disulfide bridges. Since Chinese hamster ovary (CHO) cells are widely used for the expression of recombinant proteins, we expressed recombinant human PDI (rhu PDI) in CHO cells to increase **cellular** PDI levels and examined its effect on the secretion of two different recombinant proteins: interleukin 15 (IL-15) and a tumor necrosis factor **receptor:Fc fusion protein** (TNFR:Fc). Secretion of TNFR:Fc (a disulfide-rich protein) is decreased in cells overexpressing PDI; the TNFR:Fc protein is retained inside these cells and colocalizes with the overexpressed rhu PDI protein in the endoplasmic reticulum. PDI overexpression did not result in intracellular retention of IL15. The nature of the interaction between PDI and TNFR:Fc was further investigated by expressing a disulfide isomerase mutant PDI in CHO cells to determine if the functional activity of PDI is involved in the **cellular** retention of TNFR:Fc protein.

L28 ANSWER 37 OF 53 MEDLINE on STN DUPLICATE 12

2000469052. PubMed ID: 10899034. TNFRSF1A mutations and autoinflammatory syndromes. Galon J; Aksentijevich I; McDermott M F; O'Shea J J; Kastner D L. (Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD 20892-1820, USA.) Current opinion in immunology, (2000 Aug) 12 (4) 479-86. Ref: 44. Journal code: 8900118. ISSN: 0952-7915. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The autoinflammatory syndromes are systemic disorders characterized by apparently unprovoked inflammation in the absence of high-titer autoantibodies or antigen-specific T lymphocytes. One such illness, TNF-**receptor**-associated periodic syndrome (TRAPS), presents with prolonged attacks of fever and severe localized inflammation. TRAPS is caused by dominantly inherited mutations in TNFRSF1A (formerly termed TNFR1), the gene encoding the 55 kDa TNF **receptor**. All known mutations affect the first two cysteine-rich extracellular subdomains of the **receptor**, and several mutations are substitutions directly disrupting conserved disulfide bonds. One likely mechanism of inflammation in TRAPS is the impaired cleavage of TNFRSF1A ectodomain upon **cellular** activation, with diminished shedding of the potentially antagonistic soluble **receptor**. Preliminary experience with recombinant p75 TNFR-**Fc fusion protein** in the treatment of TRAPS has been favorable.

L28 ANSWER 38 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN

2000:603983 Document No. 134:161523 Detection of membrane-bound tumor necrosis factor (TNF): An analysis of TNF-specific reagents. Gerspach, Jeannette; Gotz, Alex; Zimmermann, Gudrun; Kolle, Carmen; Bottinger, Heiner; Grell, Matthias (Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, 70569, Germany). Microscopy Research and Technique, 50(3), 243-250 (English) 2000. CODEN: MRTEEO. ISSN: 1059-910X. Publisher: Wiley-Liss, Inc..

AB Tumor necrosis factor (TNF) exists in two bioactive forms, the membrane integrated form and the proteolytically derived soluble cytokine. Both forms of TNF are involved in a variety of different physiol. and pathophysiol. situations. Here we analyzed different human and mouse TNF-specific reagents for their ability to determine the expression of membrane-expressed

TNF. The data prove some antibodies to be very useful for the anal. of transmembrane TNF expression because these antibodies distinguish between the transmembrane form of TNF and soluble TNF bound to **cellular** TNF **receptors**. In addition, we found that recombinant human TNF **receptor** fusion proteins are advantageous tools to analyze both human and mouse transmembrane TNF expression.

L28 ANSWER 39 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1999334896 EMBASE IL-2 **receptor**-targeted cytolytic IL-2/**Fc fusion protein** treatment blocks diabetogenic autoimmunity in nonobese diabetic mice. Xin Xiao Zheng; Steele A.W.; Hancock W.W.; Kawamoto K.; Xian Chang Li; Nickerson P.W.; Li Y.; Tian Y.; Strom T.B.. Dr. T.B. Strom, Department of Medicine, Division of Immunology, Beth Israel Deaconess Medical Center, P.O. Box 15707, Boston, MA 02215, United States. tstrom@caregroup.harvard.edu. Journal of Immunology 163/7 (4041-4048) 1 Oct 1999.
Refs: 27.
ISSN: 0022-1767. CODEN: JOIMA3. Pub. Country: United States. Language: English. Summary Language: English.

AB High affinity IL-2R5 is present on recently activated but not on resting or memory T cells. Selective targeting of T cells bearing high affinity IL-2R is an attractive therapy for many T cell-dependent cytopathic disease processes. A variety of rodent mAbs directed against the α-chain of the IL-2R, as well as IL-2 fusion toxins, have been used in animals and humans to achieve selective immunosuppression. Here we report on the development of a novel IL-2R targeting agent, a cytolytic chimeric IL-2/**Fc fusion protein**. This immunoligand binds specifically and with high affinity to IL-2R and is structurally capable of recruiting host Ab-dependent cell- mediated cytotoxicity and complement-dependent cytotoxicity activities. The Ig component ensures an extended circulating t(1/2) of 25 h following systemic administration. To subsequently explore the mechanisms of the antidiabetogenic effects of IL-2/Fc, we have mutated the FcR binding and complement C1q binding (Fc(-/-)) domains of the Fc fragment to render the Fc unable to direct Ab-dependent cell-mediated cytotoxicity and complement- dependent cytotoxicity activities. In a model of passive transfer of diabetes in nonobese diabetic mice, lytic IL-2/Fc, but not nonlytic IL-2/Fc(-/-), exhibited striking antidiabetogenic effects. Together with the negligible potential of IL-2/Fc for immunogenicity, this finding forecasts that cytolytic IL-2/Fc may offer a new therapeutic approach for selective targeting of auto and alloimmune T cells.

L28 ANSWER 40 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1999392121 EMBASE Molecular cloning and biological characterization of NK cell activation-inducing ligand, a counterstructure for CD48. Kubin M.Z.; Parshley D.L.; Din W.; Waugh J.Y.; Smith T.D.; Smith C.A.; MacDuff B.M.; Armitage R.J.; Chin W.; Cassiano L.; Borges L.; Petersen M.; Trinchieri G.; Goodwin R.G.. M.Z. Kubin, Immunex Corporation, 51 University Street, Seattle, WA 98101-2936, United States. mkubin@immunex.com. European Journal of Immunology 29/11 (3466-3477) 1999.
Refs: 42.
ISSN: 0014-2980. CODEN: EJIMAF. Pub. Country: Germany. Language: English. Summary Language: English.

AB Using the monoclonal antibody C1.7, which recognizes a signaling, membrane-bound molecule on human NK and a proportion of CD8+ T cells, we cloned a novel molecule we refer to as NK cell activation-inducing ligand (NAIL). It is a 365-amino acid protein that belongs to the immunoglobulin-like superfamily with closest homology to murine 2B4, and human CD84 and CD48. Using a soluble NAIL-**Fc fusion protein**, we determined the counterstructure for NAIL, CD48, which it binds with high affinity. Stimulation of human B cells with recombinant

NAIL in the presence of a suboptimal concentration of human CD40 ligand or IL-4 resulted in increased proliferation. Treatment of human dendritic cells with soluble NAIL-leucine zipper protein resulted in an increased release of IL-12 and TNF-α. Using recombinant CD48 protein, we demonstrated the ability of this molecule to increase NK cell cytotoxicity and induce IFN-γ production. We also showed that 2B4 binds to mouse CD48, suggesting that interaction of these **receptors** may play a similar role in both species. Taken together these results indicate that the NAlL-CD48 interaction may be an important mechanism regulating a variety of immune responses.

L28 ANSWER 41 OF 53 EMBASE
on STN

1999026969 EMBASE IL-13 is a key regulatory cytokine for Th2 cell-mediated pulmonary granuloma formation and IgE responses induced by Schistosoma mansoni eggs. Chiaramonte M.G.; Schopf L.R.; Neben T.Y.; Cheever A.W.; Donaldson D.D.; Wynn T.A.. Dr. T.A. Wynn, Immunobiology Section, Laboratory of Parasitic Diseases, Natl. Inst. of Allergy/Infect. Dis., 9000 Rockville Pike, Bethesda, MD 20892-0425, United States. Journal of Immunology 162/2 (920-930) 15 Jan 1999.
Refs: 64.
ISSN: 0022-1767. CODEN: JOIMA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Schistosoma mansoni egg-induced pulmonary granuloma formation is a cell-mediated inflammatory response associated with dominant Th2-type cytokine expression, tissue eosinophilia, and high levels of serum IgE. In the present study, we show that in vivo blockade of the Th2 cytokine IL-13, using soluble IL-13R α2- **Fc fusion protein**, significantly reduced the size of pulmonary granulomas in unsensitized as well as egg-sensitized mice. Blocking IL-13 also significantly reduced total serum IgE levels. Interestingly, however, IL-13 blockade did not affect the evolving egg-induced Th2-type cytokine response. IL-4, IL-5, as well as IL-13 responses were indistinguishable in control-Fc- and soluble IL-13R α2- **Fc fusion protein**-treated animals. The smaller granulomas were also phenotypically like the control Fc-treated mice, displaying a similar eosinophil content. Additional studies in IL-4-deficient mice demonstrated that IL-13 was produced, but at much lower levels than in wild-type mice, while IL-4 expression was completely independent of IL-13. Moreover, while granuloma formation was partially reduced in IL-4-deficient mice, blocking IL-13 in these animals almost completely abrogated granuloma development and the pulmonary eosinophilia, while it simultaneously increased IFN-γ, production. Together, these data demonstrate that IL-13 serves as an important mediator of Th2-mediated inflammation and plays a role in eliciting IgE responses triggered by schistosome eggs.

L28 ANSWER 42 OF 53 MEDLINE on STN

2000106263. PubMed ID: 10641509. [Rheumatoid arthritis: new molecular and **cellular** aspects]. Rheumatoide Arthritis: Neue molekulare und zellulare Aspekte. Distler O; Muller-Ladner U; Scholmerich J; Gay R E; Gay S. (Klinik und Poliklinik fur Innere Medizin I, Universitat Regensburg.) Medizinische Klinik (Munich, Germany : 1983), (1999 Dec 15) 94 (12) 673-80. Ref: 56. Journal code: 8303501. ISSN: 0723-5003. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.

AB BACKGROUND: Rheumatoid arthritis is a chronic systemic disorder of unknown etiology, that is characterized by inflammation, synovial hyperplasia and destruction of the affected joints. Novel molecular biology techniques have identified important **cellular** and molecular pathways in the pathogenesis of rheumatoid arthritis during the last years. RESULTS: The **cellular** activation of aggressively growing, matrix-degrading synovial fibroblasts is a key event in the pathogenesis of rheumatoid arthritis. The **cellular** activation results in an altered expression of apoptosis regulating molecules (for example CD 95 and

Sentrin) as well as of protooncogenes (for example RAS and MYC). Important extracellular stimuli such as the pro-inflammatory cytokines interleukin-1 and TNF-alpha are overexpressed in the rheumatoid arthritis synovium. First clinical trials with cytokine inhibiting molecules (interleukin-1 **receptor** antagonist, recombinant soluble TNF-alpha **receptor**/Etanercept and monoclonal TNF-alpha antibodies/Remicade) revealed promising results. Etanercept is now available for the treatment of rheumatoid arthritis in the USA. In addition, gene transfer methods could help to overcome the problem of a continuous expression of therapeutic molecules in the affected joints; gene delivery of the interleukin-1 **receptor** antagonist is currently tested in a human trial. Finally, the inhibition of matrix degrading enzymes such as matrix metalloproteinases, that mediate the joint destructive features of the activated synovial fibroblasts, could be another therapeutic approach. CONCLUSIONS: The elucidation of important molecular and **cellular** pathways in the pathogenesis resulted in novel concepts in the therapy of rheumatoid arthritis. Gene transfer methods are of importance in studying the pathogenesis of the disease, however, their clinical safety and usefulness have to be proven in additional studies.

L28 ANSWER 43 OF 53 MEDLINE on STN DUPLICATE 13

1999262361. PubMed ID: 10328925. Nr-CAM promotes neurite outgrowth from peripheral ganglia by a mechanism involving axonin-1 as a neuronal **receptor**. Lustig M; Sakurai T; Grumet M. (Department of Pharmacology, NYU Medical Center, 550 First Avenue, New York, New York, 10016, USA.) Developmental biology, (1999 May 15) 209 (2) 340-51. Journal code: 0372762. ISSN: 0012-1606. Pub. country: United States. Language: English.

AB Nr-CAM is a neuronal cell adhesion molecule (CAM) belonging to the immunoglobulin superfamily that has been implicated as a ligand for another CAM, axonin-1, in guidance of commissural axons across the floor plate in the spinal cord. Nr-CAM also serves as a neuronal **receptor** for several other cell surface molecules, but its role as a ligand in neurite outgrowth is poorly understood. We studied this problem using a chimeric **Fc-fusion protein** of the extracellular region of Nr-CAM (Nr-Fc) and investigated potential neuronal **receptors** in the developing peripheral nervous system. A recombinant Nr-CAM-**Fc fusion protein**, containing all six Ig domains and the first two fibronectin type III repeats of the extracellular region of Nr-CAM, retains **cellular** and molecular binding activities of the native protein. Injection of Nr-Fc into the central canal of the developing chick spinal cord in ovo resulted in guidance errors for commissural axons in the vicinity of the floor plate. This effect is similar to that resulting from treatment with antibodies against axonin-1, confirming that axonin-1/Nr-CAM interactions are important for guidance of commissural axons through a spatially and temporally restricted Nr-CAM positive domain in the ventral spinal cord. When tested as a substrate, Nr-Fc induced robust neurite outgrowth from dorsal root ganglion and sympathetic ganglion neurons, but it was not effective for tectal and forebrain neurons. The peripheral but not the central neurons expressed high levels of axonin-1 both in vitro and in vivo. Moreover, antibodies against axonin-1 inhibited Nr-Fc-induced neurite outgrowth, indicating that axonin-1 is a neuronal **receptor** for Nr-CAM on these peripheral ganglion neurons. The results demonstrate a role for Nr-CAM as a ligand in axon growth by a mechanism involving axonin-1 as a neuronal **receptor** and suggest that dynamic changes in Nr-CAM expression can modulate axonal growth and guidance during development.

L28 ANSWER 44 OF 53 EMBASE
on STN

1999019791 EMBASE Soluble vascular cell adhesion molecule (VCAM)-**Fc fusion protein** induces leukotriene C4 secretion in platelet-activating factor-stimulated eosinophils. Tsuruta R.; Cobb R.R.; Mastrangelo M.; Lazarides E.; Cardarelli P.M.. Dr. P.M. Cardarelli, Department of Biology, Tanabe Research Laboratories, 4540 Towne Centre Court, San Diego, CA 92121, United States. pcardarelli@trlusa.com. Journal of Leukocyte Biology 65/1 (71-79) 1999.
Refs: 48.
ISSN: 0741-5400. CODEN: JLBIE7. Pub. Country: United States. Language: English. Summary Language: English.

AB Eosinophil adhesion to vascular cell adhesion molecule-1 (VCAM-1) is important for **cellular** recruitment into allergic inflammatory sites. To determine whether eosinophil adhesion to VCAM-1 affects cell function, leukotriene C4 (LTC4) was measured. Human eosinophils were incubated with platelet-activating factor (PAF) in the presence or absence of soluble VCAM- **Fc fusion protein** (sVCAM-Fc) or immobilized VCAM-Fc. sVCAM-Fc induced a concentration-dependent increase in LTC4 secretion, which was dependent on the presence of PAF and not blocked by cyclic peptides shown to inhibit α4β1-dependent adhesion. Likewise, soluble ICAM-Fc induced a concentration-dependent LTC4 secretion. LTC4 secretion was induced by the calcium ionophore, A23187, and the combination of sVCAM-Fc and A23187 had synergistic properties. It is interesting to note that Mn2+ or anti-β1 monoclonal antibody, TS2/16, inhibited LTC4 secretion induced by sVCAM-Fc and PAF. Eosinophil adhesion to VCAM-Fc or interleukin-1β-stimulated endothelial cells did not induce LTC4 secretion. These data suggest that sVCAM-Fc-induced LTC4 secretion depends on distinct signals from those of eosinophil adhesion.

L28 ANSWER 45 OF 53 MEDLINE on STN DUPLICATE 14

1998184878. PubMed ID: 9516471. Construction and binding kinetics of a soluble granulocyte-macrophage colony-stimulating factor **receptor** alpha-chain-**Fc fusion protein**. Monfardini C; Ramamoorthy M; Rosenbaum H; Fang Q; Godillot P A; Canziani G; Chaiken I M; Williams W V. (Department of Medicine, Rheumatology Division, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA.) Journal of biological chemistry, (1998 Mar 27) 273 (13) 7657-67.
Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States.
Language: English.

AB Granulocyte-macrophage colony-stimulating factor (GM-CSF) activity is mediated by a **cellular receptor** (GM-CSFR) that is comprised of an alpha-chain (GM-CSFRalpha), which specifically binds GM-CSF, and a beta-chain (betac), shared with the interleukin-3 and interleukin-5 **receptors**. GM-CSFRalpha exists in both a transmembrane (tmGM-CSFRalpha) and a soluble form (sGM-CSFRalpha). We designed an sGM-CSFRalpha-**Fc fusion protein** to study GM-CSF interactions with the GM-CSFRalpha. The construct was prepared by fusing the coding region of the sGM-CSFRalpha with the CH2-CH3 regions of murine IgG2a. Purified sGM-CSFRalpha-Fc ran as a monomer of 60 kDa on reducing SDS-polyacrylamide gel electrophoresis but formed a trimer of 160-200 kDa under nonreducing conditions. The sGM-CSFRalpha-Fc bound specifically to GM-CSF as demonstrated by standard and competitive immunoassays, as well as by radioligand assay with 125I-GM-CSF. The sGM-CSFRalpha-Fc also inhibited GM-CSF-dependent cell growth and therein is a functional antagonist. Kinetics of sGM-CSFRalpha-Fc binding to GM-CSF were evaluated using an IAsys biosensor (Affinity Sensors, Paramus, NJ) with two assay systems. In the first, the sGM-CSFRalpha-Fc was bound to immobilized staphylococcal protein A on the biosensor surface, and binding kinetics of GM-CSF in solution were determined. This revealed a rapid koff of 2.43 x 10(-2)/s. A second set of experiments was performed with GM-CSF immobilized to the sensor surface and the sGM-CSFRalpha-Fc in solution. The dissociation rate constant (koff) for the sGM-CSFRalpha-Fc trimer from GM-CSF was 1.57 x 10(-3)/s, attributable to the higher avidity

of binding in this assay. These data indicate rapid dissociation of GM-CSF from the sGM-CSFRalpha-Fc and suggest that in vivo, sGM-CSFRalpha may need to be present in the local environment of a responsive cell to exert its antagonist activity.

L28 ANSWER 46 OF 53 MEDLINE on STN DUPLICATE 15
1998184542. PubMed ID: 9525630. A novel membrane protein is a mouse mammary tumor virus **receptor**. Golovkina T V; Dzuris J; van den Hoogen B; Jaffe A B; Wright P C; Cofer S M; Ross S R. (Department of Microbiology/Cancer Center, University of Pennsylvania, Philadelphia 19104-6142, USA.) Journal of virology, (1998 Apr) 72 (4) 3066-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Mouse mammary tumor virus (MMTV) infects a number of different cell types, including mammary gland and lymphoid cells, in vivo. To identify the **cellular receptor** for this virus, a mouse cDNA expression library was transfected into Cos-7 monkey kidney cells, and those transfected cells able to bind virus were selected by using antibody against the virus's cell surface envelope protein, gp52. One clone isolated from a library prepared from newborn thymus RNA, called MTVR, was able to confer virus binding to both monkey and human cells; this binding was blocked by anti-MTVR antibody. Moreover, transfection of MTVR into CV1 cells rendered them susceptible to infection by a murine leukemia virus-based retrovirus vector pseudotyped with the MMTV envelope protein. An epitope-tagged MTVR cofractionated with **cellular** membranes. Coimmunoprecipitation of the MMTV envelope protein and a MTVR-rabbit **Fc fusion protein** showed that these two proteins bound to each other. The MTVR sequence clone is unique, shows no homology to known membrane proteins, and is transcribed in many tissues.

L28 ANSWER 47 OF 53 MEDLINE on STN DUPLICATE 16
1998087604. PubMed ID: 9425167. Direct and regulated interaction of integrin alphaEbeta7 with E-cadherin. Higgins J M; Mandlebrot D A; Shaw S K; Russell G J; Murphy E A; Chen Y T; Nelson W J; Parker C M; Brenner M B. (The Lymphocyte Biology Section, Division of Rheumatology, Immunology and Allergy, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.) Journal of cell biology, (1998 Jan 12) 140 (1) 197-210. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.
AB The cadherins are a family of homophilic adhesion molecules that play a vital role in the formation of **cellular** junctions and in tissue morphogenesis. Members of the integrin family are also involved in cell to cell adhesion, but bind heterophilically to immunoglobulin superfamily molecules such as intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, or mucosal addressin cell adhesion molecule (MadCAM)-1. Recently, an interaction between epithelial (E-) cadherin and the mucosal lymphocyte integrin, alphaEbeta7, has been proposed. Here, we demonstrate that a human E-cadherin-**Fc fusion protein** binds directly to soluble recombinant alphaEbeta7, and to alphaEbeta7 solubilized from intraepithelial T lymphocytes. Furthermore, intraepithelial lymphocytes or transfected JY' cells expressing the alphaEbeta7 integrin adhere strongly to purified E-cadherin-Fc coated on plastic, and the adhesion can be inhibited by antibodies to alphaEbeta7 or E-cadherin. The binding of alphaEbeta7 integrin to cadherins is selective since cell adhesion to P-cadherin-Fc through alphaEbeta7 requires >100-fold more fusion protein than to E-cadherin-Fc. Although the structure of the alphaE-chain is unique among integrins, the avidity of alphaEbeta7 for E-cadherin can be regulated by divalent cations or phorbol myristate acetate. Cross-linking of the T cell **receptor** complex on intraepithelial lymphocytes increases the avidity of alphaEbeta7 for E-cadherin, and may provide a mechanism for the adherence and activation of lymphocytes within the epithelium in the presence of specific foreign antigen. Thus, despite its dissimilarity to known integrin ligands, the

specific molecular interaction demonstrated here indicates that E-cadherin is a direct counter **receptor** for the alphaEbeta7 integrin.

L28 ANSWER 48 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN
1997:220640 Document No. 126:208748 Cloning and expression of cDNA for herpes simplex virus **cellular** mediator HVEM and pharmaceuticals derived from the protein and cDNA. Spear, Patricia G.; Montgomery, Rebecca I. (Northwestern University, USA; Spear, Patricia G.; Montgomery, Rebecca I.). PCT Int. Appl. WO 9704658 A1 19970213, 57 pp. DESIGNATED STATES: W: CA, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US12374 19960726. PRIORITY: US 1995-509024 19950728.

AB The present invention provides isolated and purified polynucleotides that encode HVEM of mammalian origin, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making HVEM using those polynucleotides and vectors, and isolated and purified HVEM. Antisense nucleic acids based on the cDNA and HVEM or HVEM derivs. may be used in pharmaceuticals. HeLa cell cDNA encoding HVEM was cloned and sequenced. Based on the DNA sequence anal. indicating that the product is a type I membrane glycoprotein with 3.5 Cys-rich repeats, HVEM is proposed to be a new member of the tumor necrosis factor/nerve growth factor **receptor** family. CHO-K1 and CHO-ST cell lines resistant to HSV-1 entry become significantly more susceptible when expressing the HVEM cDNA. Anti-HVEM antiserum protected these cells from infection, but the mechanism was not that of preventing binding to the cells. An HVEM-**Fc fusion protein** also inhibited infection of the HVEM-producing recombinant cells.

L28 ANSWER 49 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
96:805007 The Genuine Article (R) Number: VP736. HUMAN EOSINOPHILS EXPRESS FUNCTIONAL CD30 LIGAND AND STIMULATE PROLIFERATION OF A HODGKINS-DISEASE CELL-LINE. PINTO A (Reprint); ALDINUCCI D; GLOGHINI A; ZAGONEL V; DEGAN M; IMPROTA S; JUZBASIC S; TODESCO M; PERIN V; GATTEI V; HERRMANN F; GRUSS H J; CARBONE A. IRCCS, CTR REG RIFERIMENTO ONCOL, DEPT MED ONCOL, LEUKEMIA UNIT, VIA PEDEMONTANA OCCIDENTALE, I-33081 AVIANO, ITALY (Reprint); CTR RIFERIMENTO ONCOL, DEPT MED ONCOL, I-33081 AVIANO, ITALY; CTR RIFERIMENTO ONCOL, DEPT PATHOL, I-33081 AVIANO, ITALY; UNIV ULM, MED CTR, DEPT HEMATOL & ONCOL INTERNAL MED 3, ULM, GERMANY. BLOOD (01 NOV 1996) Vol. 88, No. 9, pp. 3299-3305. ISSN: 0006-4971. Pub. country: ITALY; GERMANY. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The presence of a prominent tissue eosinophilia represents a typical histopathologic hallmark of Hodgkin's disease (HD). To evaluate the putative role of eosinophils on tumor cell regulation in HD, we have analyzed these cells for the functional expression of CD30 ligand (CD30L), a surface molecule able to transduce CD30-mediated proliferation signals on Hodgkin's (H) and Reed-Sternberg (RS) cells. The results demonstrate that circulating and tissue eosinophils from normal donors and patients with HD or hypereosinophilic syndrome (HES), display CD30L mRNA and express CD30L protein, as shown by immunostaining with a specific monoclonal antibody (M80) and with a biotinylated soluble CD30-**Fc fusion protein**. The surface density of CD30L on eosinophils from HD and HES patients was remarkably higher compared with healthy donors, probably reflecting a cytokine-mediated upregulation in these pathologic conditions. Accordingly, we provide evidence that cytokines regulating eosinophils proliferation and activation, ie, interleukin-5 (IL-5), IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF), are able to enhance the **cellular** density of CD30L on purified eosinophils from normal subjects. Finally, we show that native CD30L on human eosinophils is a functionally active surface structure able to transduce proliferative signals on CD30(+) target cells, including cultured H-RS cells. Our data suggest that eosinophils may not

merely represent innocent bystanders, but rather act as important elements in the pathology of HD by contributing to the deregulated network of CD30/CD30L-mediated interactive signals between H-RS cells and surrounding reactive cells. (C) 1996 by The American Society of Hematology.

L28 ANSWER 50 OF 53 MEDLINE on STN

1999035154. PubMed ID: 9816306. Phase I trial of interleukin 2 in combination with the soluble tumor necrosis factor **receptor** p75 IgG chimera. Trehu E G; Mier J W; Dubois J S; Sorce D; Klempner M S; Epstein M; Dinarello C A; Shapiro L; Kappler K; Ronayne L; Atkins M B. (Divisions of Hematology-Oncology and Infectious Diseases, Tufts University School of Medicine and New England Medical Center Hospitals, Boston, Massachusetts 02111, USA.) Clinical cancer research : an official journal of the American Association for Cancer Research, (1996 Aug) 2 (8) 1341-51. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB Our purpose was to determine the effective biological dose and/or maximum tolerated dose of recombinant human tumor necrosis factor **receptor** :IgG chimera (rhuTNFR:Fc; Immunex, Seattle, WA) in combination with interleukin 2 (IL-2) with regard to reduction in IL-2 toxicity and modulation of biological effects of high-dose IL-2 administration. Twenty-four patients with metastatic cancer were treated with escalating doses of rhuTNFR:Fc at 1, 1, 5, 10, and 20 mg/m2 i.v. on days 1 and 15 (dose levels 1-5) or 10, 20, and 30 mg/m2 days 1 and 15 plus 50% dose on days 3, 5, 17, and 19 (dose levels 6-8) prior to IL-2 at doses of 300,000 IU/kg (dose level 1) and 600,000 IU/kg (dose levels 2-8) i.v. every 8 h on days 1-5 and 15-19. The t1/2 of rhuTNFR in patients receiving IL-2 was 72 h. The median number of IL-2 doses was 24, and central nervous system, skin, and cardiac arrhythmias were the major dose-limiting toxicities. TNF bioactivity was inhibited, and the polymorphonuclear leukocyte chemotactic defect normally seen with IL-2 was not observed. Increases in C-reactive protein, IL-6, IL-8, and IL-1 **receptor** antagonist levels were partially suppressed relative to historical controls, whereas peripheral blood mononuclear cell phenotypes, urinary nitrate, endothelial adhesion molecule expression in skin biopsies, and **cellular** infiltrates in tumor biopsies were consistent with findings in patients treated with IL-2 alone. Four patients developed thyroid dysfunction. There were five responses: two complete responses (both melanoma) and three partial responses (response rate, 21%). rhuTNFR:Fc may modulate the toxicity and some of the biological effects of IL-2 while preserving antitumor activity. Dose level 6 (10 mg/m2 on days 1 and 15, and 5 mg/m2 on days 3, 5, 17, and 19) has been chosen for a randomized, double-blind, placebo-controlled trial of IL-2 with and without rhuTNFR:Fc.

L28 ANSWER 51 OF 53 CAPLUS COPYRIGHT 2004 ACS on STN

1995:950285 Document No. 123:336062 ST2/T1 protein functionally binds to two secreted proteins from Balb/c 3T3 and human umbilical vein endothelial cells but does not bind interleukin 1. Kumar, Sanjay; Minnich, Michael D.; Young, Peter R. (Dep. Mol. Immunol. Protein Biochem., SmithKline Beecham Res. Dev., King of Prussia, PA, 19406, USA). Journal of Biological Chemistry, 270(46), 27905-13 (English) 1995. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Bio logy.

AB The ST2/T1 **receptor**, a homolog of the interleukin 1 **receptor** (IL-1R), was expressed in COS and Drosophila S2 cells as a human IgG-**Fc fusion protein**. While a type I IL-1RFc fusion protein bound human IL-1 in vitro, the ST2Fc fusion protein did not. Furthermore, IL-1 stimulated a synthetic interleukin-8 promoter reporter gene that was cotransfected into Jurkat cells with a full-length IL-1R type I (IL-1R1) or a chimeric **receptor** composed of the IL-1RI extracellular domain and ST2 intracellular domain. In contrast, IL-1 did not stimulate the interleukin-8 promoter when cotransfected with a full-length ST2 or an ST2 extracellular/IL-1R

intracellular domain fusion protein. Both IL-1RI and the IL-1R/ST2R chimeric **receptor** also activated a **receptor**-associated kinase and CSBP/p38 MAP kinase. Using ST2Fc **receptor**, we have identified, through **receptor** precipitation, **receptor**-dot blot and surface plasmon resonance, a putative ligand of ST2 secreted from Balb/c 3T3 and human umbilical vein endothelial cells. The putative ligand was also able to stimulate CSBP/p38 MAP kinase through the ST2 **receptor**. These results suggest that the ST2 is not an IL-1 **receptor** but rather has its own cognate ligand.

L28 ANSWER 52 OF 53 MEDLINE on STN DUPLICATE 17

95363081. PubMed ID: 7543517. Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. Steurer W; Nickerson P W; Steele A W; Steiger J; Zheng X X; Strom T B. (Harvard Medical School, Department of Medicine, Boston, MA, USA.) Journal of immunology (Baltimore, Md. : 1950), (1995 Aug 1) 155 (3) 1165-74. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB To test the hypothesis that blockade of B7-triggered costimulation by donor cells could preclude allograft rejection, we coated crude islet allograft preparations in vitro for 1 h with a murine CTLA4/**Fc fusion protein**. Murine CTLA4/Fc blocks the proliferative response in primary mixed lymphocyte cultures (MLC) and Con A-stimulated murine spleen cell cultures by 85 to 95%. Responder cells from a primary MLC containing mCTLA4/Fc were hyporesponsive upon restimulation to the same stimulator cells in a secondary MLC lacking mCTLA4/Fc. Because of mutations in the Fc gamma RI and C'1q binding sites of the Fc portion of the murine CTLA4/**Fc fusion protein**, the molecule binds to, but does not target, cells for Ab-dependent **cellular** cytotoxicity or complement-directed cytolysis. Although systemic immunosuppression was not applied, 42% (10 of 24) of B6AF1 recipients of islet allografts pretreated with CTLA4/Fc were permanently engrafted. Further, 50% of hosts bearing functioning islet allografts more than 150 days post-transplant were formally proved to be tolerant to donor tissues. A persistent CD4+ and CD8+ T cell infiltrate surrounding, but not invading, islet grafts in tolerant hosts was discerned. In control experiments, 89% (8 of 9) of islet allografts coated with mIgG3, and 100% (n = 10) pretreated with media alone were rejected. Thus, we conclude that 1) B7-triggered costimulation by donor APCs is an important element of rejection, and 2) blockade of the B7 pathway by in vitro allograft manipulation is able to induce tolerance.

L28 ANSWER 53 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

1993:525493 Document No.: PREV199396138900. Induction of circulating and erythrocyte-bound IL-8 by IL-2 immunotherapy and suppression of its in vitro production by IL-1 **receptor** antagonist and soluble tumor necrosis factor **receptor** (p75) chimera. Tilg, Herbert; Shapiro, Leland; Atkins, Michael B.; Dinarello, Charles A.; Mier, James W. [Reprint author]. Div. Hematol.-Oncol., Dep. Med., New England Med. Center, 750 Washington St., Box 245, Boston, MA 02111, USA. Journal of Immunology, (1993) Vol. 151, No. 6, pp. 3299-3307.
CODEN: JOIMA3. ISSN: 0022-1767. Language: English.

AB The objective of this study was 1) to investigate the in vivo production of IL-8 in patients undergoing IL-2 immunotherapy and 2) to study the influence of IL-1Ra, soluble TNF **receptor** p75 (TNFsRp75), and a TNFsRp75-**Fc fusion protein** on IL-2-induced IL-8 production in vitro. Circulating IL-8 was assessed both in plasma and erythrocyte lysates prepared from patients undergoing IL-2 immunotherapy. IL-8 was detectable in the plasma within 2-4 h after the first IL-2 infusion, reached a peak level after 4 h, and declined rapidly to undetectable within 8 h. Erythrocyte-bound IL-8 was also detected within 4 h of the first IL-2 dose, but levels were higher than those measured in plasma and remained elevated long after the plasma levels had become undetectable. On day 4 of therapy, the increases in both plasma

and the erythrocyte-lysate IL-8 levels induced by an IL-2 injection were less pronounced than on day 1. Although IL-1Ra and TNFsRp75-Fc individually had only a modest suppressive effect on IL-2-induced IL-8 production by PBMC in vitro, the combination of IL-1Ra and TNFsRp75-Fc markedly down-regulated IL-2-induced IL-8 synthesis and steady-state mRNA levels. TNFsRp75 had no effect on IL-2-induced IL-8 synthesis. Our studies suggest that the transient detection of IL-8 in plasma early in the course of IL-2 treatment is due to erythrocyte sequestration and that suppressed synthesis, due in part to high levels of circulating IL-1 and TNF antagonists, may play a role later in the course of treatment.

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L31 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:435073 Document No. 139:21018 **Fusion** proteins of biologically active peptides and antibodies as vaccines. **Kohler, Heinz** (USA). U.S. Pat. Appl. Publ. US 2003103984 A1 20030605, 10 pp., Cont.-in-part of U.S. 6,238,667. (English). CODEN: USXXCO. APPLICATION: US 2001-865281 20010529. PRIORITY: US 1998-70907 19980504.

AB The invention provides a **fusion** protein made up of (1) an antibody and (2) a peptide having a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and homophilic activities wherein the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody. In the present invention that is accomplished by a method comprising the steps of creating a **fusion** gene including a nucleic acid sequence encoding an antibody and a nucleic acid sequence encoding the peptide, wherein the nucleic acid sequence encoding the peptide is located inside the nucleic acid sequence encoding the antibody at a site wherein, when the **fusion** is expressed, the **fusion** protein created thereby comprises the antibody and the peptide, wherein the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody. The invention also provides a composition and a pharmaceutical composition comprising a **fusion** protein of a peptide with a biol. activity selected from the group consisting of immunostimulatory, membrane transport, and homophilic activities and an antibody. An example is presented of enhancing the activity of an anti-idiotypic vaccine consisting of an anti-idiotypic antibody (murine anti-idiotypic antibody 3H1 which mimics the carcinoembryonic antigen) via crosslinking it with a peptide derived from the complement C3d region 1217-1232.

L31 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2002:927546 Document No. 138:23668 **Fusion** proteins of antibodies and biologically active peptides with increased therapeutic value and their preparation. **Kohler, Heinz**; Morgan, Charles (Immpheron, Inc., USA; Innexus Corporation). PCT Int. Appl. WO 2002097041 A2 20021205, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,

UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US16651 20020529. PRIORITY: US 2001-865281 20010529.

AB **Fusion** proteins of antibodies and peptides having a biol. activity such as immunostimulation, membrane transport and homophilic activities in which the peptide is connected to the antibody at a site that does not interfere with antigen binding of the antibody are described for therapeutic use. The protein is manufactured by expression of a cloned gene in which the peptide coding sequence is inserted into the antibody gene at a site where the peptide will not interfere with the antigen binding properties of the antibody. The peptide may be flanked by loop-forming or conformation-conferring sequences. The use of a peptide derived from complement C3 to improve the antigenicity of a monoclonal antibody to carcinoembryonic antigen is demonstrated.

L31 ANSWER 3 OF 15 MEDLINE on STN DUPLICATE 1

2001086866. PubMed ID: 11114926. Transcriptional organization and dynamic expression of the hbpCAD genes, which encode the first three enzymes for 2-hydroxybiphenyl degradation in Pseudomonas azelaica HBP1. Jaspers M C; Schmid A; Sturme M H; Goslings D A; **Kohler H P**; Roelof Van Der Meer J. (Swiss Federal Institute for Environmental Science and Technology and Swiss Federal Institute of Technology, CH-8600 Dubendorf, Switzerland.) Journal of bacteriology, (2001 Jan) 183 (1) 270-9. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB Pseudomonas azelaica HBP1 degrades the toxic substance 2-hydroxybiphenyl (2-HBP) by means of three enzymes that are encoded by structural genes hbpC, hbpA, and hbpD. These three genes form a small noncontiguous cluster. Their expression is activated by the product of regulatory gene hbpR, which is located directly upstream of the hbpCAD genes. The HbpR protein is a transcription activator and belongs to the so-called XylR/DmpR subclass within the NtrC family of transcriptional activators. Transcriptional **fusions** between the different hbp intergenic regions and the luxAB genes of Vibrio harveyi in P. azelaica and in Escherichia coli revealed the existence of two HbpR-regulated promoters; one is located in front of hbpC, and the other one is located in front of hbpD. Northern analysis confirmed that the hbpC and hbpA genes are cotranscribed, whereas the hbpD gene is transcribed separately. No transcripts comprising the entire hbpCAD cluster were detected, indicating that transcription from P(hbpC) is terminated after the hbpA gene. E. coli mutant strains lacking the structural genes for the RNA polymerase sigma(54) subunit or for the integration host factor failed to express bioluminescence from P(hbpC)- and P(hbpD)-luxAB **fusions** when a functional hbpR gene was provided in trans. This pointed to the active role of sigma(54) and integration host factor in transcriptional activation from these promoters. Primer extension analysis revealed that both P(hbpC) and P(hbpD) contain the typical motifs at position -24 (GG) and -12 (GC) found in sigma(54)-dependent promoters. Analysis of changes in the synthesis of the hbp mRNAs, in activities of the 2-HBP pathway enzymes, and in concentrations of 2-HBP intermediates during the first 4 h after induction of continuously grown P. azelaica cells with 2-HBP demonstrated that the specific transcriptional organization of the hbp genes ensured smooth pathway expression.

L31 ANSWER 4 OF 15 MEDLINE on STN DUPLICATE 2

2000096684. PubMed ID: 10629187. HbpR, a new member of the XylR/DmpR subclass within the NtrC family of bacterial transcriptional activators, regulates expression of 2-hydroxybiphenyl metabolism in Pseudomonas azelaica HBP1. Jaspers M C; Suske W A; Schmid A; Goslings D A; **Kohler H P**; van der Meer J R. (Swiss Federal Institute for Environmental Science and Technology, CH-8600 Dubendorf, Switzerland.) Journal of bacteriology, (2000 Jan) 182 (2) 405-17. Journal code: 2985120R. ISSN:

0021-9193. Pub. country: United States. Language: English.

AB The regulation of 2-hydroxybiphenyl and 2,2'-dihydroxybiphenyl degradation in Pseudomonas azelaica is mediated by the regulatory gene, hbpR. The hbpR gene encodes a 63-kDa protein belonging to the NtrC family of prokaryotic transcriptional activators and having the highest homology to members of the XylR/DmpR subclass. Disruption of the hbpR gene in P. azelaica and complementation in trans showed that the HbpR protein was the key regulator for 2-hydroxybiphenyl metabolism. Induction experiments with P. azelaica and Escherichia coli containing luxAB-based transcriptional **fusions** revealed that HbpR activates transcription from a promoter (P(hbpC)) in front of the first gene for 2-hydroxybiphenyl degradation, hbpC, and that 2-hydroxybiphenyl itself is the direct effector for HbpR-mediated activation. Of several compounds tested, only the pathway substrates 2-hydroxybiphenyl and 2,2'-dihydroxybiphenyl and structural analogs like 2-aminobiphenyl and 2-hydroxybiphenylmethane were effectors for HbpR activation. HbpR is therefore, to our knowledge, the first regulator of the XylR/DmpR class that recognizes biaromatic but not monoaromatic structures. Analysis of a spontaneously occurring mutant, P. azelaica HBP1 Prp, which can grow with the non-wild-type effector 2-propylphenol, revealed a single mutation in the hbpR gene (T613C) leading to a Trp-->Arg substitution at amino acid residue 205. P. azelaica HBP1 derivative strains without a functional hbpR gene constitutively expressed the genes for 2-hydroxybiphenyl degradation when complemented in trans with the hbpR-T613C gene. This suggests the importance of this residue, which is conserved among all members of the XylR/DmpR subclass, for interdomain repression.

L31 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1999:216944 Document No. 130:236462 Method of affinity crosslinking biologically active, immunogenic peptides to antibodies. **Kohler, Heinz** (USA). PCT Int. Appl. WO 9914244 A1 19990325, 33 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US19710 19980918. PRIORITY: US 1997-59515 19970919.

AB A method of affinity crosslinking a peptide to an antibody by photo-chemical activating an azido compound in a peptide comprising said azido compound; adding an antibody to the photochem. activated peptide; and allowing the photochem. activated peptide and the antibody to react. The azido compound has an affinity for a hydrophobic structure in the variable domain of the antibody which binds to nucleotides or nucleosides, binding the peptide into a native binding pocket of the Ig (Ig) structure of an antibody. The site of crosslinking is located away from the antigen binding site in the Fv domain avoiding the compromise of antigen recognition. A composition of a peptide cross-linked to an antibody is also disclosed. Thus, anti-idiotype vaccines were prepared by crosslinking 3H1, an anti-idiotype antibody that mimics carcinoembryonic antigen, or 38C13, an anti-idiotype antibody of B lymphoma, with a C3d peptide (i.e. KNRWEDPGKQLYNVEA) to enhance antigen presentation.

L31 ANSWER 6 OF 15 MEDLINE on STN DUPLICATE 3

1998341068. PubMed ID: 9676379. [**Fusion** of the craniocervical transition with "CerviFix" after survived atlanto-occipital dislocation]. Die **Fusion** des craniocervicalen Ubergangs mit dem "CerviFix" nach uberlebter atlanto-occipitaler Dislokation. **Kohler H**; Vock B; Hochstein P; Wentzensen A. (Berufsgenossenschaftliche Unfallklinik Ludwigshafen.) Der Chirurg; Zeitschrift fur alle Gebiete der operativen Medizen, (1998 Jun) 69 (6) 677-81. Journal code: 16140410R. ISSN: 0009-4722. Pub. country: GERMANY: Germany, Federal Republic of. Language:

German.

AB We report a rare case of a young moped rider who suffered atlanto-occipital dislocation. He survived with tetraplegia. We discuss the accident mechanism, the anatomical particulars, and the clinical features and compare our operative treatment with the cases described in the literature.

L31 ANSWER 7 OF 15 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

1998:363378 The Genuine Article (R) Number: ZL948. Enhanced molecular mimicry of CEA using photoaffinity crosslinked C3d peptide. Lou D Y; **Kohler H (Reprint)**. IMMPHERON INC, LEXINGTON, KY 40509 (Reprint); IMMPHERON INC, LEXINGTON, KY 40509; UNIV KENTUCKY, DEPT MICROBIOL & IMMUNOL, LEXINGTON, KY 40536. NATURE BIOTECHNOLOGY (MAY 1998) Vol. 16, No. 5, pp. 458-462. Publisher: NATURE PUBLISHING CO. 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707. ISSN: 1087-0156. Pub. country: USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Antigen mimicry of using anti-idiotypic antibodies for use as cancer vaccines has been disappointing due to the weak immunogenicity of immunoglobulin variable domains. To enhance the immunogenicity of an anti-idiotype vaccine we incorporated a molecular adjuvant peptide into the antibody. The peptide is derived from the C3d region known to bind CR2 receptors on B-cells. A photoreactive peptide is synthesized that affinity-labels a single site in the antibody variable domain. The molecular adjuvant peptide is crosslinked to the anti-idiotype mimetic by chemical means without modifying other sites on the antibody. The C3d-conjugated anti-idiotype antibody induces a strong idiotype and antigen-specific response in mice.

L31 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1996:513778 Document No. 125:165687 Recombinant monoclonal anti-idiotype antibody 3H1 sequences relating to human carcinoembryonic antigen and use in vaccine. Chatterjee, Malaya; **Kohler, Heinz**; Chatterjee, Sunil K.; Foon, Kenneth A. (University of Kentucky, USA). PCT Int. Appl. WO 9620277 A2 19960704, 120 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US17103 19951228. PRIORITY: US 1994-365484 19941228.

AB This invention provides compns. derived from the sequences encoding the variable light and/or variable heavy regions of monoclonal anti-idiotype antibody 3H1 and methods for using these compns.

L31 ANSWER 9 OF 15 MEDLINE on STN DUPLICATE 4

97045030. PubMed ID: 8881034. Recognition by human Vgamma9/Vdelta2 T cells of melanoma cells upon **fusion** with Daudi cells. Viale O; van der Bruggen P; Meuer E; Kunzmann R; **Kohler H**; Mertelsmann R; Boon T; Fisch P. (Department of Medicine I, Freiburg University Medical Center, Hugstetter Str. 55, D-79106 Freiburg, Germany.) Immunogenetics, (1996) 45 (1) 27-34. Journal code: 0420404. ISSN: 0093-7711. Pub. country: United States. Language: English.

AB Daudi Burkitt's lymphoma cells, unlike other tumor cell lines, stimulate human T cells coexpressing the variable (V) region genes TCRG-V9 and V TCRD-V2 to proliferate and secrete lymphokines. Hybrids, derived by the **fusion** of Daudi cells with the human melanoma cell line MZ2-MEL 2.2, retain the morphology of melanoma cells. Unlike the parental melanoma cell line, these Daudi x MZ2-MEL 2.2 hybrids stimulate secretion of tumor necrosis factor (TNF) and granulocyte/macrophage colony stimulating factor (GM-CSF) by CD4-positive Vgamma9/Vdelta2 T-cell clones. Whereas the stimulator phenotype of Daudi cells behaves as a dominant trait in Daudi x melanoma hybrids, the expression of B-cell differentiation markers is suppressed. Thus, the gamma/delta T-cell

ligand expressed by Daudi cells behaves as a dominant tumor antigen in Daudi x melanoma hybrids and is unrelated to the differentiated B-cell phenotype. Dominant expression of the Daudi ligand for human Vgamma9/Vdelta2 T cells in these hybrids may provide a basis for defining the stimulatory principle at the molecular level.

L31 ANSWER 10 OF 15 MEDLINE on STN DUPLICATE 5

93020189. PubMed ID: 1403647. Clonal dominance: cause for a limited and failing immune response to HIV-1 infection and vaccination. **Kohler H**; Goudsmit J; Nara P. (San Diego Regional Cancer Center, California.) Journal of acquired immune deficiency syndromes, (1992) 5 (11) 1158-68. Ref: 80. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB Oligoclonal and monoclonal antibody populations against different HIV-encoded proteins are common in sera from healthy HIV-1-infected individuals. This is especially important when it includes functional antibody repertoires directed at neutralizing cell free virus or inhibiting cell **fusion** of virus-infected cells. In the host, during the acute viral syndrome following HIV-1 infection, a rapidly replicating, cell-free and genotypically homogeneous viral population is known to arise from the transmitted viral inoculum. Dominant B and possibly T cell clones responsible for both functional and nonfunctional antibodies appear to arise early in response to this initially homogeneous cell-free viral population heralding seroconversion. During the viremic phase, deposition of cell-free virus as either complement coated or as immune complexes (iccosomes) within the germinal centers results in continued and long-term boosting of primed B cells. This saturation of antigen presenting germinal centers and the presence of limited, immunodominant cross-reactive epitopes on the envelope glycoprotein of the closely-related and immune selected viral quasispecies in the host appear to continue the boosting effect of the primed secondary response. This repertoire freeze appears to be responsible for limiting the recruitment of new uncommitted B cells to other functional epitopes or affinity maturation of B-cell clones to escape variants and the subsequent production and quality of functional antibody against the evolving/selected virus populations. This may include in addition to neutralizing and cell **fusion** inhibiting antibody, direct complement-fixing and/or NK-directed antibody-dependent cell-mediated antibody as well as various effector, helper, or T cell-mediated activity. In addition to antiviral antibody responses, antibody directed to other invading pathogens or opportunistic organisms may also be clonaly restricted. Antibody facilitating infectivity or blocking effective immunity may also be included in this phenomena and thus be over represented by such a mechanism. AIDS vaccines utilizing the envelope must identify these epitopes to avoid creating clonal dominance and therefore possibly limit the breadth and specificity of a humoral response following infection. Furthermore, immunotherapeutic approaches designed to recruit humoral immune effector function must be able to overcome the dominance of noneffective antibodies and restore a normal polyclonal immune response against HIV. Further research, therefore, into the humoral and cellular dysregulating properties of the HIV-1 envelope is warranted.

L31 ANSWER 11 OF 15 MEDLINE on STN

91130440. PubMed ID: 1825196. [The immunobiology of transplant rejection and its pharmacological modification]. Immunbiologie der Transplantatabstossung und deren pharmakologische Beeinflussung. **Kohler H**; Zanker B; Strom T B. (I. Medizinische Klinik und Poliklinik, Universitat Mainz.) Deutsche medizinische Wochenschrift, (1991 Feb 15) 116 (7) 264-9. Ref: 25. Journal code: 0006723. ISSN: 0012-0472. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.

L31 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 6

87281660. PubMed ID: 3497200. Idiotype vaccines against human T cell acute lymphoblastic leukemia. I. Generation and characterization of biologically active monoclonal anti-idiotopes. Bhattacharya-Chatterjee M; Pride M W; Seon B K; **Kohler H.** Journal of immunology (Baltimore, Md. : 1950), (1987 Aug 15) 139 (4) 1354-60. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB A murine monoclonal anti-tumor antibody termed SN2 (Ab1), isotype IgG1-kappa, that defines a unique human T cell leukemia-associated cell-surface glycoprotein, gp37 (m.w. 37,000), was used to generate monoclonal anti-idiotype antibodies (Ab2) in syngeneic BALB/c mice. The Ab2 were screened on the basis of their binding to the F(ab')2 fragments of SN2 and not to the F(ab')2 of pooled normal BALB/c mice sera IgG1 or to an unrelated BALB/c monoclonal antibody of the same isotype. Fifteen Ab2, obtained from two **fusions**, were specific for the SN2 idiotope and not against isotype or allotype determinants. To find out whether these Ab2 are directed against the paratope of SN2, the binding of radiolabeled SN2 to leukemic MOLT-4 and JM cells which contain gp37 as a surface constituent was studied in the presence of these anti-idiotopes. Clone 4EA2 inhibited the binding 100% at a concentration of 50 ng and 4DC6 inhibited 90% at a concentration of 250 ng. A third clone 4DD6 gave about 50% inhibition. Similar was the inhibition of SN2 binding to insolubilized MOLT-4 antigen or cell membrane preparation. The binding of SN2 (Ab1) to 4EA2 and 4DC6 was also inhibited by semipurified preparation of gp37 antigen. These results demonstrate that at least two of the anti-idiotope antibodies are binding either at or near the binding site idiotope of SN2. Next, the purified Ab2 was used to immunize syngeneic mice to induce antibody binding to MOLT-4 cells or gp37. Sera from mice immunized with 4EA2 and 4DC6 coupled to keyhole limpet hemocyanin contained antibodies which bind to semipurified gp37 antigen and MOLT-4 cells. Immune sera inhibited the binding of iodinated Ab2 and Ab1 indicating that an anti-anti-idiotopic antibody (Ab3) in mice shares idiotopes with Ab1 (SN2). Also, the binding of iodinated Ab2 to Ab1 was inhibited by rabbit antisera specific for gp37. Collectively, these data suggest that anti-idiotype antibodies 4EA2 and 4DC6 may be useful in the generation of idiotype vaccines against human T cell leukemia.

L31 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 7

86142527. PubMed ID: 3950546. Immunoglobulin with complementary paratope and idiotope. Kang C Y; **Kohler H.** Journal of experimental medicine, (1986 Apr 1) 163 (4) 787-96. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB A hybridoma antibody (11E7-1) was isolated from a myeloma **fusion** with nu/nu BALB/c immunized against the T15 idiotype. This IgM antibody exhibited a dual specificity, binding both to PC and to anti-PC antibodies from two idiotype families. Binding to PC and anti-PC antibodies are completely inhibited by PC analogs. Furthermore, the hybridoma antibody binds to itself. Self-binding is also inhibited by PC analogs. From these data, we suggest that 11E7-1 hybridoma antibody has a PC-specific paratope site, and at same time expresses the internal PC antigen idiotope. The term autobody is proposed to signify its self-binding and potential role in autoimmunity. Autobodies may have a unique role in the network of immune system. Furthermore, it may be a model for designing idiotype vaccines.

L31 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 8

85113008. PubMed ID: 6084243. Amniotic band disruption syndrome associated with elevated amniotic AFP and normal acetylcholinesterase gel test. Aitken D A; May H M; Ferguson-Smith M A; Howat R; **Kohler H G.** Prenatal diagnosis, (1984 Nov-Dec) 4 (6) 443-6. Journal code: 8106540. ISSN: 0197-3851. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Ultrasonography in an 18 week pregnancy selected for detailed scanning because of a single elevated maternal serum AFP result suggested the

presence of anencephaly. Amniocentesis at 19 weeks yielded a clear AF with an elevated AFP result (5.4 MOM) and no evidence of an acetylcholinesterase band of neural origin on PAG electrophoresis. At termination, the fetus had cephalo-amniotic **fusion** and multiple abnormalities including bilateral cleft lip and palate and digital amputations characteristic of the amniotic band syndrome. The cranial defect was completely sealed by attachment of the amniotic surface of the placenta to the skull.

L31 ANSWER 15 OF 15 MEDLINE on STN DUPLICATE 9
82099422. PubMed ID: 7033381. Immune response to phosphorylcholine. IX. Characterization of hybridoma anti-TEPC15 antibodies. Wittner M K; Bach M A; **Kohler H.** Journal of immunology (Baltimore, Md. : 1950), (1982 Feb) 128 (2) 595-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Hybridoma antibodies against the PC-binding T15 BALB/c myeloma protein were raised by cell **fusion** with anti-T15 A/He immune cells. The idiotype specificity of these monoclonal anti-T15 antibodies was determined with a panel of different myeloma and hybridoma immunoglobulins. Two types of anti-T15 antibodies are seen. One reacts with a number of different IgA myeloma proteins and with serum IgA of certain strains of mice; this reactivity most likely is due to allotypy. The other group consists of anti-T15 antibodies that are specific for the T15 idiotype and are therefore termed anti-idiotypic. The bindings of the anti-idiotype antibodies to T15 were specifically inhibited by T15 (F(ab')2 but not by other PC-binding myeloma proteins of different idiotypes. The relationship of the idiotype-specific anti-T15 antibodies to the PC-binding site of the T15 idiotype was analyzed by hapten inhibition of anti-idiotypic binding and by inhibition of BALB/c anti-PC splenic hemolytic plaque formation. Anti-T15 antibodies, for which the T15 binding is inhibited by PC or PC-BSA, also specifically inhibit anti-PC plaque formation. These antibodies are labeled site and near-site anti-idiotypic antibodies. Site and near-site-specific anti-idiotypic antibodies recognize different idiotopes on the T15 molecules. The possible differential biologic activities of these anti-idiotopes in idiotype network regulation is considered.

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---Logging off of STN---

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Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	293.80	294.01

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-28.41	-28.41

STN INTERNATIONAL LOGOFF AT 17:05:57 ON 04 MAY 2004